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(54) **PROCESS FOR MAKING ANTIFUSOGENIC FUSION PEPTIDES THAT FORM INCLUSION BODIES**

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(57) **ABSTRACT**

A process is disclosed for the production of an antifusogenic peptide by producing a fusion peptide of a length of about 14 to 70 amino acids in a prokaryotic host cell, comprising the steps, under such conditions that inclusion bodies of said fusion peptide are formed, of: (a) expressing in said host cell a nucleic acid encoding said fusion peptide consisting of a first peptide which is an antifusogenic peptide of a length of about 10 to 50 amino acids and a second peptide of a length of about 4 to 30 amino acids, said first peptide being N-terminally linked to said second peptide; (b) cultivating said host cell to produce said inclusion bodies; and (c) recovering said antifusogenic peptide from said inclusion bodies, wherein said recovered antifusogenic peptide consists of said fusion peptide or a peptide comprising the antifusogenic peptide of about 10 to 50 amino acids and which is a fragment cleaved from said fusion peptide. Inclusion bodies of the peptides are disclosed. Also disclosed is a nucleic acid encoding the fusion peptide consisting of a first peptide which is an antifusogenic peptide selected from the group of SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, and said sequences further consisting of glycine at the C terminal end, N-terminally linked to the second peptide.

IB9
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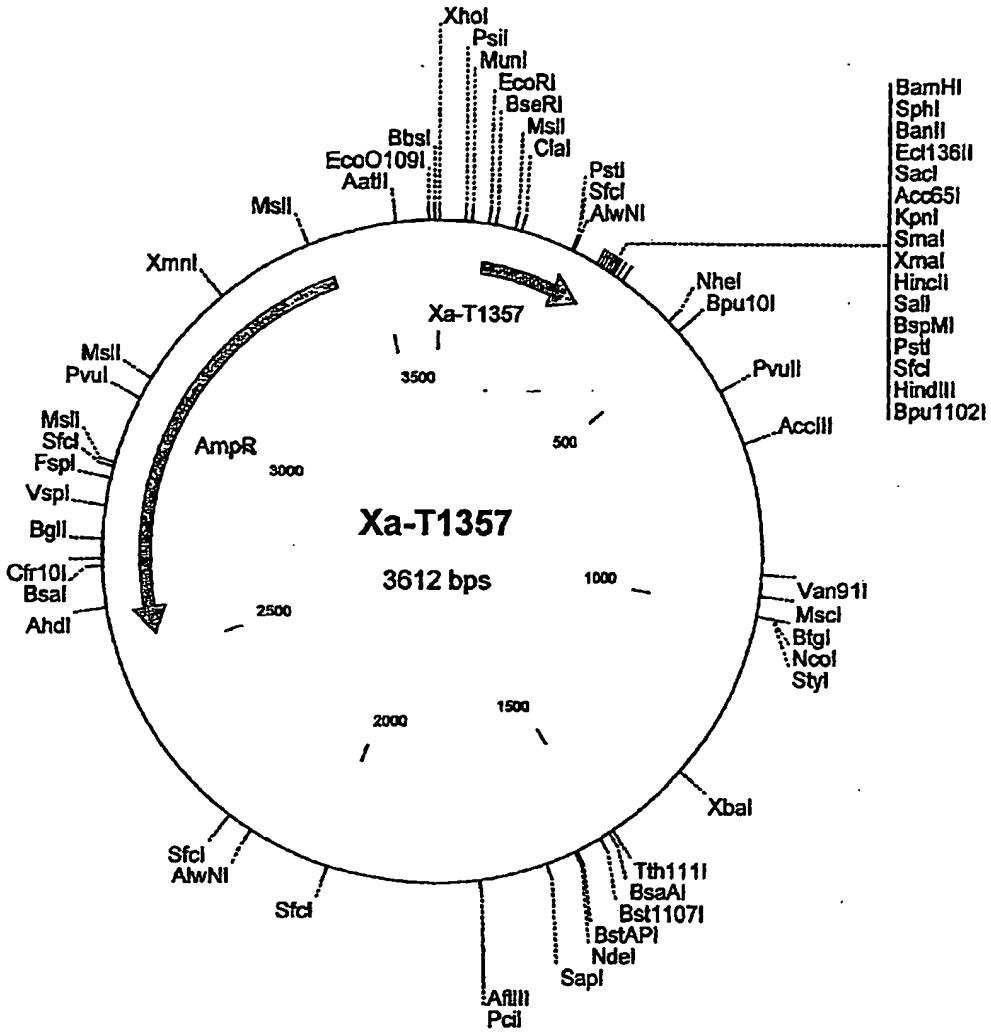
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PROCESS FOR MAKING ANTIFUSOGENIC FUSION PEPTIDES THAT FORM INCLUSION BODIES

FIELD OF THE INVENTION

[0001] The invention relates to methods for the recombinant production of peptides which inhibit the fusion of viruses with membranes of target cells. In particular, this invention relates to the recombinant production of peptidic inhibitors of lentivirus such as human immunodeficiency virus (HIV), Simian immunodeficiency virus (SIV), measles virus (MEV), influenza viruses such as respiratory syncytical virus (RSV) or human parainfluenza virus (HPV).

BACKGROUND OF THE INVENTION

[0002] Fusion of viruses with cellular membranes is an essential step for the entry of enveloped viruses, such as HIV-I, HIV-II, RSV, measles virus, influenza virus, parainfluenza virus, Epstein-Barr virus and hepatitis virus, into cells. After having entered the cell the cascade of viral replication may be initiated resulting in viral infection.

[0003] HIV is a member of the lentivirus genus, which includes retroviruses that possess complex genomes and exhibit cone-shaped capsid core particles. Other examples of lentiviruses include the simian immunodeficiency virus (SIV), visna virus, and equine infectious anemia virus (EIAV). Like all retroviruses, HIV's genome is encoded by RNA, which is reverse-transcribed to viral DNA by the viral reverse transcriptase (RT) upon entering a new host cell. Influenza viruses and their cell entry mechanisms are described by Bullough, P. A., et al., Nature 371 (1994) 37-43; Carr, C. M., and Kim, P. S., Cell 73 (1993) 823-832; and Wilson, I. A., et al., Nature 289 (1981) 366-373.

[0004] All lentiviruses are enveloped by a lipid bilayer that is derived from the membrane of the host cell. Exposed surface glycoproteins (SU, gp120) are anchored to the virus via interactions with the transmembrane protein (TM, gp41). The lipid bilayer also contains several cellular membrane proteins derived from the host cell, including major histocompatibility antigens, actin and ubiquitin (Arthur, L. O., et al., Science 258 (1992) 1935-1938). A matrix shell comprising approximately 2000 copies of the matrix protein (MA, p17) lines the inner surface of the viral membrane, and a conical capsid core particle comprising ca. 2000 copies of the capsid protein (CA, p24) is located in the center of the virus. The capsid particle encapsidates two copies of the unspliced viral genome, which is stabilized as a ribonucleoprotein complex with ca. 2000 copies of the nucleocapsid protein (NC, p7), and also contains three essential virally encoded enzymes: protease (PR), reverse transcriptase (RT) and integrase (IN). Virus particles also package the accessory proteins, Nef, Vif and Vpr. Three additional accessory proteins that function in the host cell, Rev, Tat and Vpu, do not appear to be packaged.

[0005] In the case of HIV, viral entry is associated with the HIV envelope surface glycoproteins (Lawless, M. K., et al., Biochemistry 35 (1996) 13697-13708; and Turner, B. G., and Summers, M. F., J. Mol. Biol. 285 (1999) 1-32). In the case of HIV-I, this surface protein is synthesized as a single 160 kD precursor protein, which is cleaved by a cellular protease into two glycoproteins gp-41 and gp-120. gp-41 is a transmembrane protein, and gp-120 is an extracellular protein which remains non-covalently associated with gp-41 in a trimeric or multimeric form (Hammarskjöld, M.-L., et al., Biochim. Biophys. Acta 989 (1989) 269-280). HIV is targeted to $CD4^+$ lymphocytes because the CD4 surface protein acts as the cellular receptor for the HIV-I virus. Viral entry into cells is dependent upon gp-120 binding to the cellular $CD4^+$ receptor molecules while gp-41 anchors the envelope glycoprotein complex in the viral membrane and mediates membrane fusion (McDougal, J. S., et al., Science 231 (1986) 382-385; and Maddon, P. J., et al., Cell 47 (1986) 333-348).

[0006] gp41 is the transmembrane subunit that mediates fusion of viral and cellular membranes. The gp41 ectodomain core is a six-helix bundle composed of three helical hairpins, each consisting of an N helix paired with an antiparallel C helix (Chan, D. C., et al., Cell 89 (1997) 263-273; Weissenhorn, W., et al., Nature 387 (1997) 426-430; Tan, K., et al., Proc. Natl. Acad. Sci. USA 94 (1997) 12303-12308). The N helices form an interior, trimeric coiled coil with three conserved, hydrophobic grooves; a C helix packs into each of these grooves. This structure likely corresponds to the core of the fusion-active state of gp41. According to Chan, D. C., et al., Proc. Natl. Acad. Sci. USA 95 (1998) 15613-15617, there is evidence that a prominent cavity in the coiled coil of the HIV type 1 gp41 is an attractive drug target.

[0007] It is assumed that the mechanism by which gp-41 mediates membrane fusion may involve the formation of a coiled-coil trimer, which is thought to drive the transition from resting to fusogenic states, as is described, for example, for influenza hemagglutinin (Wilson, I. A., et al., Nature 289 (1981) 366-373; Carr, C. M., and Kim, P. S., Cell 73 (1993) 823-832; Bullough, P. A., et al., Nature 371 (1994) 37-43).

[0008] C peptides (peptides corresponding to the C helix) of enveloped viruses, such as DP178 and C34, potently inhibit membrane fusion by both laboratory-adapted strains and primary isolates of HIV-1 (Malashkevich, V. N., et al., Proc. Natl. Acad. Sci. USA 95 (1998) 9134-9139; Wild, C. T., et al., Proc. Natl. Acad. Sci. USA 91 (1994) 9770-9774). A Phase I clinical trial with the C peptide DP178 suggests that it has antiviral activity in vivo, resulting in reduced viral loads (Kilby, J. M., et al., Nature Medicine 4 (1998) 1302-1307). The structural features of the gp41 core suggest that these peptides act through a dominant-negative mechanism, in which C peptides bind to the central coiled coil of gp41 and lead to its inactivation (Chan, D. C., et al., Cell 93 (1998) 681-684).

[0009] Within each coiled-coil interface is a deep cavity, formed by a cluster of residues in the N helix coiled coil, that has been proposed to be an attractive target for the development of antiviral compounds. Three residues from the C helix (Trp-628, Trp-631, and Ile-635) insert into this cavity and make extensive hydrophobic contacts. Mutational analysis indicates that two of the N-helix residues (Leu-568 and Trp-571) comprising this cavity are critical for membrane fusion activity (Cao, J., et al., J. Virol. 67 (1993) 2747-2755). Therefore, compounds that bind with high affinity to this cavity and prevent normal N and C helix pairing may be effective HIV-1 inhibitors. The residues in the cavity are highly conserved among diverse HIV-1 isolates. Moreover, a C peptide containing the cavity-binding region is much less susceptible to the evolution of resistant

virus than DP178, which lacks this region (Rimsky, L. T., et al., J. Virol. 72 (1998) 986-993). These observations suggest that high-affinity ligands targeting the highly conserved coiled-coil surface, particularly its cavity, will have broad activity against diverse HIV isolates and are less likely to be bypassed by drug-escape mutants.

[0010] Fusogenic structures of envelope fusion proteins was shown from influenza, Moloney murine leukemia virus, and simian immunodeficiency virus (cit. in Chan, D. C., Proc. Natl. Acad. Sci. USA 95 (1998) 15613-15617), human respiratory syncytial virus, Ebola, human T cell leukemia virus, simian parainfluenza. It indicates a close relationship between the families of orthomyxoviridae, paramyxoviridae, retroviridae, and others like filoviridae, in which viral entry into target cells is enabled by like transmembrane glycoproteins such as gp41 of HIV-1, hemagglutinin of influenza, GP2 of Ebola and others (Zhao, X., et al., Proc. Natl. Acad. Sci. USA 97 (2000) 14172-14177).

[0011] In the state of the art, methods are described for the preparation of peptidic inhibitors (C-peptides) (see, e.g., Root, M. J., et al., Science 291 (2001) 884-888; Root et al. describe peptide C37-H6 which is derived from HIV-1. HXB2 and contains residues 625-661. It was recombinantly expressed as N40-segement with a GGR-linker and a histidine tag, expressed in *E.coli* and purified from the soluble fraction of bacterial lysates. Zhao, X., et al. describe in Proc. Natl. Acad. Sci. USA 97 (2000) 14172-14177 a synthetic gene of recRSV-1 (human respiratory syncytial virus) which encodes Residues 153-209, a G-rich linker, residues 476-524, Factor Xa cleavage site and a his-tag. Chen, C. H., et al., describe in J. Virol. 67 (1995) 3771-3777 the recombinant expression of the extracellular domain of gp41 synthesized as fusion protein, residues 540-686, fusion to MBP.

[0012] A number of peptidic inhibitors, also designated as antifusogenic peptides, of such membrane fusion-associated events are known, including, for example, inhibiting retroviral transmission to uninfected cells. Such peptides are described, for example, by Lambert, D. M., et al., Proc. Natl. Acad. Sci. USA 93 (1996) 2186-2191, in U.S. Pat. Nos. 6,013,263; 6,017,536; and 6,020,459; and in WO 00/69902, WO 99/59615 and WO 96/40191. Further peptides inhibiting fusing associated events are described, for example, in U.S. Pat. Nos. 6,093,794; 6,060,065; 6,020,459; 6,017,536; 6,013,263; 5,464,933; 5,656,480; and in WO 96/19495.

[0013] Examples of linear peptides derived from the HIV-I gp-41 ectodomain which inhibit viral fusion are DP-107 and DP-178. DP-107 is a portion of gp-41 near the N-terminal fusion peptide and has been shown to be helical, and it strongly oligomerizes in a manner consistent with coiled-coil formation (Gallaher, W. R., et al., Aids Res. Hum. Retrovirus 5 (1989) 431-440, Weissenhorn, W., et al., Nature 387 (1997) 426-430). DP-178 is derived from the C-terminal region of the gp-41 ecto-domain. (Weissenhorn, W., et al., Nature 387 (1997) 426-430). Although without discernible structure in solution this peptide and constrained analogs therefrom adopt a helical structure, bind to a groove of the N-terminal coiled-coil trimer of gp41 and thus prevent the gp41 to transform into the fusogenic state (Judice, J. K., et al., Proc. Natl. Acad. Sci. USA 94 (1997) 13426-13430).

[0014] Such short-chain peptides usually are prepared by chemical synthesis. Chemical synthesis is described, for example, by Mergler, M., et al., Tetrahedron Letters 29 (1988) 4005-4008 and 4009-4012; Andersson, L., et al., Biopolymers 55 (2000) 227-250; and by Jones, J. H., J. Pept. Sci. 6 (2000) 201-207. Further methods are described in WO 99/48513.

[0015] However, chemical peptide synthesis suffers from several drawbacks. Most important is racemization, which results in insufficient optical purity. In peptide chemistry, racemization also means epimerization at one of several chirality centers. If only 1% racemization occurs for a single coupling step, then at 100 coupling steps only 61% of the target peptide would be received (Jakubke, H. D., Peptide, Spektrum Akad. Verlag, Heidelberg (1996), p. 198). It is obvious that the number of impurities increases with growing chain length and their removal is more and more difficult and costly.

[0016] Chemical synthesis on large scale is limited by high costs and lack of availability of protected amino acid derivatives as starting materials. On the one hand, these starting materials should be used in excess to enable complete reactions, on the other hand, their use should be balanced for cost reasons, safety and environmental aspects (Andersson et al., Biopolymers 55 (2000) 227-250).

[0017] Peptides may also be produced by recombinant DNA technology. Whereas recombinant production of soluble proteins of chain lengths of more than 50 amino acids is known from the state of the art, the production of peptides with fewer than 50 amino acids suffers from several drawbacks (Doebeli, H., et al, Protein Expression and Purification 12 (1998) 404-414). Such short or medium chain peptides are usually not stably expressed. They are attacked by intrinsic peptidases and degraded. This may result from their small size or lack of highly ordered tertiary structure (WO 00/31279). Other authors have found that recombinant production of peptides requires a minimum chain length of 60 to 80 amino acids for a stable expression, and it is further common knowledge that such peptides are produced as soluble peptides and not as inclusion bodies (see, e.g., van Heeke, G., et al., Methods in Molecular Biology 36 (1994) 245-260, eds. B. M. Dunn and M. W. Pennington, Humana Press Inc., Totowa, N.J.); and Goldberg et al., Maximizing Gene Expression (1986), pp. 287-314, eds. Reznikoff, W., and Gold, L., Butterworks, Stoneham, Mass.). Recombinant production of shorter peptides is especially not successful because if such peptides are expressed in prokaryotes, they remain soluble and are immediately degraded by prokaryotic peptidases. To avoid this problem, according to common knowledge, such peptides are expressed as large (more than 150 to 200 amino acids) fusion proteins, whereby the fusion tail either renders the fusion protein fairly soluble and avoids the formation of inclusion bodies or the fusion tail is a protein which forms during recombinant expression in prokaryotes, inclusion bodies, and therefore fusion protein consisting of such fusion tail and the desired short peptide will also form inclusion bodies during overexpression in prokaryotes. A great disadvantage of such methods is that the molecular weight of the fusion tail is considerably higher than the molecular weight of the desired peptide. Therefore, the yield of the desired peptide is very low and the excess of cleaved fusion tail has to be separated of.

[0018] Lepage, P., et al., in Analytical Biochemistry 213 (1993) 40-48, describe recombinant methods for the production of HIV-1Rev peptides. The peptides are expressed

as fusion proteins with the synthetic immunoglobulin type G (IgG) binding domains of Staphylococcus aureus protein A. The peptides have a length of about 20 amino acids, whereas the IgG-binding part has a length of about 170 amino acids, so that the expressed fusion protein has an overall length of about 190 amino acids. This fusion protein is expressed, secreted in soluble form in the medium, and purified by affinity chromatography. The authors reported that with this method it might be possible to produce recombinant protein in an amount of hundreds of milligrams per liter of culture. However, this methodology is limited due to alternative processing within the signal peptide sequence and several post-translational modifications of the fused proteins as well as of the cleaved peptides. Assuming an average molecular weight of an amino acid of 110 Daltons, the desired peptides have a molecular weight of about 2,000 to 5,000 Daltons, whereas the fusion tail has a length of at least 170 amino acids (about 19,000 D), if the IgG binding domains of Staphylococcus aureus protein A is used as such a fusion tail. Therefore, only 10 to 25% of the recombinantly produced protein is the desired peptide.

[0019] EP 0 673 948 describes the recombinant production of a gp41 peptide as a fusion protein with β-galactosidase using the expression vector pSEM3 (Knapp, S., et al., BioTechniques 8 (1990) 280-281). This fusion protein contains a large part of β-galactosidase gene, encoding the N-terminal 375 amino acids and additional 23 codons of a polylinker sequence.

[0020] Further examples and methods for the recombinant production of small peptides via large fusion proteins in *E.coli* are described by Uhlen and Moks, "Gene Fusions For Purposes of Expression, An Introduction" in Methods in Enzymology 185 (1990) 129-143, Academic Press. In regard to the production via the "inclusion body" way, Uhlen and Moks refer to large fusion products involving fusion parts like trpE, cII and again β-galactosidase. Ningyi, L., et al., Gaojishu Tongxun 10 (2000) 28-31 describe the recombinant expression of p24 gag gene in *E.coli*.

SUMMARY OF THE INVENTION

[0021] The present invention provides a process for the production of an antifusogenic peptide by producing a fusion peptide of a length of about 14 to 70 amino acids in a prokaryotic host cell, comprising the steps, under such conditions that inclusion bodies of said fusion peptide are formed, of:

[0022] a) expressing in said host cell a nucleic acid encoding said fusion peptide consisting of a first peptide which is an antifusogenic peptide of a length of about 10 to 50 amino acids and a second peptide of a length of about 4 to 30 amino acids, said first peptide being N-terminally linked to said second peptide;

[0023] b) cultivating said host cell to produce said inclusion bodies; and

[0024] c) recovering said antifusogenic peptide from said inclusion bodies, wherein said recovered antifusogenic peptide consists of said fusion peptide or a peptide comprising the antifusogenic peptide of about 10 to 50 amino acids resulting from cleavage of said fusion peptide.

[0025] The present invention also provides a nucleic acid encoding a fusion peptide of from about 14 to 70 amino acids consisting of a first peptide which is an antifusogenic peptide selected from the group of SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, and said sequences further consisting of glycine at the C terminal end, N-terminally linked to a second peptide consisting of 4 to 30 amino acids.

[0026] In addition, the present invention provides a composition comprising inclusion bodies consisting essentially of at least one fusion peptide of from about 14 to 70 amino acids consisting of a first peptide which is an antifusogenic peptide selected from the group of SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, and said sequences further consisting of glycine at the C terminal end, N-terminally linked to a second peptide consisting of 4 to 30 amino acids, and optionally at least one peptide comprising the antifusogenic peptide of from about 10 to 50 amino acids and which is a fragment of said fusion peptide.

[0027] It is an object of the present invention to provide a method for the recombinant production of a high yield of antifusogenic peptides via the inclusion body route. It is also an object of the invention to provide such a method which is suitable for the large-scale industrial production of antifusogenic peptides.

BRIEF DESCRIPTION OF THE FIGURE

[0028] **FIG. 1** shows the expression vector encoding Xa-T 1357.

BRIEF DESCRIPTION OF THE SEQUENCES

[0029] SEQ ID NO:1 Synthetic gene Xa-T 1357.

[0030] SEQ ID NO:2 Peptide Xa-T1357.

[0031] SEQ ID NO:3 Primer 1.

[0032] SEQ ID NO:4 Primer 2.

[0033] SEQ ID NO:5 Primer 3.

[0034] SEQ ID NO:6 Primer 4.

[0035] SEQ ID NO:7 Peptide T1357.

[0036] SEQ ID NO:8 Peptide T680.

[0037] SEQ ID NO:9 Peptide RSV118.

[0038] SEQ ID NO:10 Peptide MV257.

[0039] SEQ ID NO:11 Peptide Xa-RSV118.

[0040] SEQ ID NO:12 Peptide Xa-MV257.

[0041] SEQ ID NO:13 Peptide M-HHHHHH-AIDV-IEGR-T1357-G.

[0042] SEQ ID NO:14 Peptide M-HHHHHH-IEGR-T1357-G.

[0043] SEQ ID NO:15 Peptide M-HHHHHH-IEGR-T680-G.

[0044] SEQ ID NO:16 Cleavage sequence.

[0045] SEQ ID NO:17 Cleavage sequence.

[0046] SEQ ID NO:18 Cleavage sequence.

[0047] SEQ ID NO:19 Cleavage sequence.

DETAILED DESCRIPTION OF THE INVENTION

[0048] The present invention provides a process for the production of an antifusogenic peptide as a fusion peptide of a length of about 14 to 70 amino acids in a prokaryotic host cell, characterized in that, under such conditions that inclusion bodies of said fusion peptide are formed,

[0049] a) in said host cell there is expressed a nucleic acid encoding said fusion peptide consisting of said antifusogenic peptide of a length of about 10 to 50 amino acids N-terminally linked to a further peptide of a length of about 4 to 30 amino acids;

[0050] b) said host cell is cultivated;

[0051] c) said inclusion bodies are formed, recovered and solubilized;

[0052] d) said fusion peptide is isolated.

[0053] Preferably said antifusogenic peptide is cleaved off from said further peptide during or after solubilization of the inclusion bodies.

[0054] Preferably, the antifusogenic peptide is a fragment of the C helix of a transmembrane subunit of an envelope fusion protein from a virus of the lentivirus genus.

[0055] Said further peptide, if expressed recombinantly in said host cell as a non-fusion peptide, would not be found in the form of inclusion bodies since it is very short.

[0056] In a preferred embodiment of the invention, the further peptide consists of a first and/or second part,

[0057] (a) wherein said first part is a stretch of from 1 to 20 amino acids, preferably hydrophilic amino acids influencing the isoelectric point of the fusion peptide, and/or said first part differing significantly from the antifusogenic peptide with regard to solubility, interactions with chromatographic separation resins and/or improves access of cleavage proteases (Polyak, S. W., et al., Protein Eng. 10 (1997) 615-619) and

[0058] (b) wherein said second part is a cleavable peptidic linker region from 1 to 10 amino acids (see, e.g., Table 3) and is located adjacent to the N-terminus of the fusogenic peptide and the C-terminus of the first part.

[0059] If the further peptide consists only of the first or the second part, said part has a length of at least 4 amino acids (including start codon coded methionine) preferably for the purpose of stabilizing during expression mRNA. Said 4 amino acids include preferably at least one arginine.

[0060] In a further embodiment of the invention, the antifusogenic peptide contains a glycine attached to its C-terminus. This glycine is useful for the purpose of enzymatic C-terminal amidation.

[0061] In a preferred embodiment of the invention, the ratio of the molecular weight of the antifusogenic peptide to the molecular weight of the further peptide in the fusion peptide is 10:1 to 1:2.

[0062] Further preferred embodiments of the invention comprise a prokaryotic expression vector containing a nucleic acid encoding a fusion peptide according to the invention and its use for the recombinant production of said peptides.

[0063] Preferred embodiments of the invention include a preparation of inclusion bodies of a fusion peptide according to the invention and methods for the production of such inclusion bodies.

[0064] A further preferred embodiment of the invention is a nucleic acid which encodes a fusion peptide according to the invention.

[0065] It was surprisingly found that short-chain antifusogenic peptides (preferably of a length of 10 to 50, more preferably of a length of 10 to 40 amino acids) can be expressed successfully as short fusion peptides of a length of up to 70 amino acids in prokaryotes such as *E.coli* via the inclusion body route.

[0066] According to the invention, a fusion peptide consisting of an antifusogenic peptide N-terminally linked to a further peptide is overexpressed in prokaryotes under conditions, whereby insoluble protein inclusion bodies are formed. Inclusion bodies are found in the cytoplasm if an expression vector which does not contain a signal sequence is used, which otherwise might enable soluble secretion of the protein into the periplasm or the medium. These inclusion bodies are separated from other cell components, for example by centrifugation after cell lysis. The inclusion bodies are solubilized by denaturing agents such as guanidine hydrochloride, urea, substances such as arginine, or strong bases such as KOH or NaOH. After solubilization, such proteins usually have to be refolded by dilution or by dialysis. As the fusion peptide produced according to the method of the invention is a short-chain peptide without disulfide bridges, renaturation is not necessary. After solubilization, the solution conditions, such as the pH, etc. are simply modified in such a way that cleavage of the further peptide is possible, and cleavage is performed if requested. The fusion peptide or antifusogenic peptide can then be recovered from this solution in a simple fashion, for example by size exclusion chromatography, ion exchange chromatography or reverse phase chromatography.

[0067] "Antifusogenic" and "anti-membrane fusion" as used herein refer to a peptide's ability to inhibit or reduce the level of fusion events between two or more structures, e.g., cell membranes or viral envelopes or pili relative to the level of membrane fusion which occurs between the structures in the absence of the peptide. Examples hereof are peptidic inhibitors of lentiviruses such as human immunodeficiency virus (HIV), respiratory syncytical virus (RSV), human parainfluenza virus (HPV), measles virus (MEV), and Simian immunodeficiency virus (SIV). Such antifusogenic peptides are derived from C helix of a transmembrane subunit of an envelope fusion protein from a virus of the lentivirus genus and bind to the central coiled coil of the transmembrane subunit of the respective virus.

[0068] Especially preferred are HIV-1 antifusogenic peptides. Table 1 describes examples of HIV-1 antifusogenic peptides derived from the C-peptide of gp41. These antifusogenic peptides and fragments thereof are particularly useful in the invention.

TABLE 1

Name*	Name	Amino acid sequence (one-letter code)	SEQ ID NO:
T-1249	T1357[1]	WQEWEQKITALLEQAQIQQEKNEYELQKLDKW ASLWEWF	7
T-20, DP178	T680[2]	YTSLIHSLIEESQNQQEKNEQELLELDKWASL WNWF	8
T-118	RSV118[3]	FDASISQVNEKINQSLAFIRKSDELLHNVNAG KST	9
T-257	MV257[3]	LHRIDLGPPISLERLDVGTNLGNAIAKLEDAK ELL	10

*for N-terminally acetylated and/or C-terminally amidated peptide; T-20 (synonymous with DP178) and T-1249 are from human immunodeficiencyvirus type 1 (HIV-1), T-118 is from respiratory syncytial virus (RSV) and T-257 is from measles virus (MV)

[1]WO 99/59615

[2]Rimsky, L. T., et al., J. Virol. 72 (1998) 986–993

[3]Lambert, D. M., Proc. Nad. Acad. Sci. USA 93 (1996) 2186–2191

[0069] The further peptide according to the invention is a peptide which is added N-terminally to the antifusogenic peptide not for purposes of improving the formation of inclusion bodies. Its purpose is to, for example, improve expression mRNA stabilization, purification (e.g. a His-tag; see, e.g., Zhang, J.-H., et al., Nanjing Daxue Xuebao, Ziran Kexue 36(4) (2000) 515-517) or to allow subsequent N-terminal modification like acetylation or PEGylation.

[0070] The further peptide according to the invention is a short peptidic stretch consisting of at least four amino acids (methionine and three further amino acids for cleavage mRNA stabilization and/or expression purposes) to about 30 amino acids preferably to about 20 amino acids (with regard to the nucleic acid codons level). The length of the further peptide is not critical for the invention as long as this peptide is a peptide which would be formed in soluble form in the cytoplasm during recombinant expression in prokaryotes under conditions where large proteins such as immunoglobulins, streptavidin or tissue-type plasminogen activator are produced as inclusion bodies. However, it is preferred that the further peptide is very short for improving the yield of the antifusogenic peptide. (If the ratio of length of further peptide to antifusogenic peptide is 3:1, the amount of antifusogenic peptide in relation to the amount of the fusion peptide recovered is about 50% lower than if the ratio is 1:1 if the same amount of fusion peptide is produced.) The length of the further peptide is selected such that the total length of the fusion peptide does not exceed about 70 amino acids, preferably 50 and most preferably 40 amino acids, for reasons of obtaining acceptable yields of antifusogenic peptides. Therefore, it is especially preferred that the further peptide consists only of an appropriate cleavage site, of some amino acids for improving expression and solubility of the fusion peptide, facilitating the solubilization process of its inclusion bodies or to improve access of cleavage proteases (avoidance of steric hindrance) and/or of some amino acids such as a His-tag for purification means (Hengen, P., Trends Biochem. Sci. 20 (1995) 285-286) and methionine necessary and encoded by the start codon.

[0071] The further peptide is a short-chain peptide which, if overexpressed alone in prokaryotes such as *E.coli* and without a signal sequence, would not be formed as inclusion bodies but remains soluble in the cytoplasm or is rapidly degraded in the cytoplasm. Such proteins do not form a fixed denatured tertiary structure, therefore they cannot form a fixed tertiary structure which is poorly soluble, therefore remain soluble and would be degraded by *E.coli* proteases if expressed.

[0072] The further peptide according to the invention consists preferably of amino acids which do not form a fixed tertiary conformation which might sterically hinder access of the cleavage agent to the cleavage site between the antifusogenic peptide and the further peptide. For this reason, the further peptide is preferably free of cysteine residues. Cysteine contains a sulfhydryl or thiol group which is highly reactive and can form disulfide bonds. The presence of cysteine can work against the desired lack of fixed secondary or tertiary conformation of the further peptide and therefore its use is avoided.

[0073] In a preferred embodiment of the present invention, the N-terminally attached further peptide stretch contains a sequence at its C-terminus, which is cleavable easily by enzymatic or chemical means.

[0074] The further peptide according to the invention is used in the fusion peptide also preferably for protection of the N-terminus of the antifusogenic peptide during expression, solubilization, purification and peptide modification. Such fusion peptides are especially valuable in a process for the production of N-terminally modified antifusogenic peptides. Such a method involves forming the recombinant polypeptide as a fusion peptide, which fusion part protects the N-terminus. The recombinant fusion peptide can then be reacted with up to three chemical protecting agents to selectively protect reactive side-chain groups and thereby prevent side-chain groups from being modified. Then the fusion peptide is cleaved with at least one cleavage reagent between the peptide and the fusion part to form an unprotected terminal amino acid reactive group. The unprotected terminal amino acid reactive group is modified with at least one chemical modifying agent such as acetic anhydride or acetic N-hydroxysuccinimide ester for N-terminal acetylation. The side-chains are then deprotected to form a N-terminally modified peptide. Such methods are described, for example, in WO 94/01451; U.S. Pat. No. 5,635,371; and U.S. Pat. No. 5,656,456.

[0075] The further peptide part preferably has such a structure that it facilitates the purification of the fusion peptide. The further peptide preferably contains for this purpose an "affinity tag" (cf. Pandjaitan, B., et al., Gene 237 (1999) 333-342), such as polyhistidine (about 6 His residues) or the like.

[0076] It is preferred to select the further peptide in such a way that its isoelectric point (IP) differs from the IP of the antifusogenic peptide for easy expression and separation of the fragments after cleavage. Table 2 shows IP's of different fusion peptides and the IP's of non-fusion fusogenic peptides T680 and T1357 (nomenclature according to WO 96/40191 and WO 99/59615). Preferably, the IP of the fusion peptide and the antifusogenic portion of the fusion peptide differ by at least about one pH unit, preferably by about 1 to 2 pH units. Such IP shift can preferably performed by basic amino acids and/or histidines contained in the further peptide.

TABLE 2

Calculating the isoelectric points of antifusogenic peptides

SEQ ID NO:	Sequence[2)]	Calculated
8	*T680*	4.13
15	M-HHHHHH-IEGR-*T680*-G	6.13
–	*T1249*	5.1[1)]
7	*T1357*	4.53
14	M-HHHHHH-IEGR-*T1357*-G	6.23
2	MRGS-HHHHHH-AIVD-IEGR-*T1357*-G	6.23

[1)]Experimentally determined.
[2)]Amino acids in one-letter code; inverse parts are peptide names.

[0077] In a further preferred embodiment of the invention, the antifusogenic peptide contains a glycine at its C-terminus. This glycine is useful for the purpose of subsequent enzymatic C-terminal amidation (Bradbury, A. F., and Smyth, D. G., Trends Biochem. Sci. 16 (1991) 112-115).

[0078] Especially preferred fusion peptides according to the invention are (amino acids in standard one-letter code):

MRGS-HHHHHH-AIDV-IEGR-*T1357*-G, (SEQ ID NO:2)

MRGS-HHHHHH-AIDV-IEGR-*RSV118*-G (SEQ ID NO:11)

MRGS-HHHHHH-AIDV-IEGR-*MV257*-G (SEQ ID NO:12)

M-HHHHHH-AIDV-IEGR-*T680*-G, (SEQ ID NO:13)

M-HHHHHH-IEGR-*T1357*-G (SEQ ID NO:14)

[0079] said fusion peptides without the poly HIS tag (His 6), or with the first four amino acids of SEQ ID NO:2, 11 or 12 as further peptide in the fusion peptides.

[0080] Annotation: The inverse parts are peptide names, and therefore the letters included in these parts do not constitute one-letter codes for amino acids.

[0081] There exist a large number of publications which describe the recombinant production of proteins in prokaryotes via the inclusion body route. Examples of such publications are Misawa, S., et al., Biopolymers 51 (1999) 297-307; Lilie, H., Curr. Opin. Biotechnol. 9 (1998) 497-501; Hockney, R. C., Trends Biotechnol. 12 (1994) 456-463.

[0082] The fusion peptides according to the invention are overexpressed in prokaryotes. Overexpression without a signal peptide leads to the formation of inclusion bodies. Methionine coded by the start codon and mentioned in the examples above can be cleaved during further processing. General methods for overexpression of proteins in prokaryotes have been well-known in the state of the art for a long time. Examples of publications in the field are Skelly, J. V., et al., Methods Mol. Biol. 56 (1996) 23-53 and Das, A., Methods Enzymol. 182 (1990) 93-112.

[0083] Overexpression in prokaryotes means expression using optimized expression cassettes with promoters such as the tac or lac promoter (EP-B 0 067 540). Usually, this can be performed by the use of vectors containing chemical inducible promoters or promoters inducible via shift of temperature. One of the useful promoters for *E.coli* is the temperature-sensitive λPL promoter (cf. EP-B 0 041 767). A further efficient promoter is the tac promoter (cf. U.S. Pat. No. 4,551,433). Such strong regulation signals for prokaryotes such as *E.coli* usually originate from bacteria-challenging bacteriophages (see Lanzer, M., et al., Proc. Natl. Acad. Sci. USA 85 (1988) 8973-8977; Knaus, R., and Bujard, H., EMBO Journal 7 (1988) 2919-2923; for the λT7 promoter Studier, F. W., et al., Methods Enzymol. 185 (1990) 60-89); for the T5 promoter EP 0 186 069).

[0084] By the use of such overproducing prokaryotic cell expression systems the fusion peptides according to the invention are produced at levels at least comprising 10% of the total expressed protein of the cell, and typically 30-40%, and occasionally as high as 50%.

[0085] "Inclusion bodies" ((IBs) as used herein refers to an insoluble form of polypeptides recombinantly produced after overexpression of the encoding nucleic acid in prokaryotes. This phenomenon is widely known in the state of the art and is reviewed, for example, by Misawa S., and Kumagai, I., Biopolymers 51 (1999) 297-307); Guise, A. D., et al., Mol. Biotechnol. 6 (1996) 53-64; and Hockney et al., Trends Biotechnol. 12 (1994) 456-463.

[0086] Solubilization of the inclusion bodies is preferably performed by adding denaturing agents such as urea, guanidine hydrochloride or alkaline solutions of KOH or NaOH (Guise, A. D., et al., Mol. Biotechnol. 6 (1996) 53-64; Fischer, B., et al., Arzneimittelforschung 42 (1992) 1512-1515).

[0087] The fusion peptides according to the invention can be cleaved enzymatically after solubilization with a specifically cleaving protease (restriction protease). The proteinase is selected, taking into consideration the amino acid sequence of the antifusogenic peptide to be produced. Care must be taken that, if possible, the recognition/cleavage sequence of the restriction proteinase does not occur in the antifusogenic peptide, and preferably also not in the further peptide, i.e., it should only occur once in the cleavage region (linker region). Suitable specifically cleaving endoproteinase are, for example, Factor Xa, thrombin, subtilisin, BTN variant/ubiquitin protease peptidase, rennin, collagenase, trypsin, chymotrypsin, endoproteinase Lys-C, kallikrein (Carter, P.: In: Ladisch, M. R.; Willson, R. C.; Painton, C. C.; Builder, S. E., eds., Protein Purification: From Molecular Mechanisms to Large-Scale Processes; ACS Symposium Series No. 427, American Chemical Society, pp. 181-193 (1990)), TEV proteinase (Parks, T. D., et al., Anal. Biochem. 216 (1994) 413-417), IgA protease (Pohlner, J., et al., Nature 325 (1987) 458-462), Kex2p proteinase (EP-A 0 467 839) or S. aureus V8 proteinase.

[0088] In addition to being cysteine-free, the sequence of the further peptide may preferably exploit other design strategies which promote efficient cleavage in the preselected cleavage environment. Particularly if the preselected cleavage agent is an endopeptidase, it is preferred that the further peptide is soluble in aqueous environments. Amino acids having charged side-groups and hydrophilic properties

are, therefore, preferably included in the further peptide to promote solubility or any other amino acids which promote the access of cleavage proteases. Such amino acids are, for example, Glu and Asp (anionic), Arg and Lys, and Ser and Thr (neutral, hydrophilic). If arginine is used, it must be taken into account that arginine constitutes the trypsin cleavage site. Therefore, in such cases where the further peptide should contain arginine, trypsin should be avoided as cleaving protease. The use of lysine, too, is subject to limitations. If the peptide has to be N-terminally modified (see above), there may be a need for protecting lysine groups. Therefore, in such cases, it is preferred to avoid lysine in the further peptide.

[0089] The cleavage site is typically selected so that it is not contained in the antifusogenic peptide and is preferably contained in the further peptide. Chemical and enzymatic cleavage sites and the corresponding agents used to effect cleavage of a peptide bond close to one of the sites are described, for example, in WO 92/01707 and WO 95/03405.

[0090] Examples for cleavage enzymes and the cleavage sequence are described in Table 3 below:

TABLE 3

Enzyme	Cleavage sequence[1)]	SEQ ID NO:
Enterokinase	DDDDK	16
Factor Xa	IEGR	17
Thrombin	RGPR	18
Ubiquitin	RGG	
Rennin	HPFHL-LVY	19
Trypsin	D or R	
Chymotrypsin	F or Y or W	
Clostripain	R	
S. aureus V8	G	

Chemical cleavage:

Cleavage substance	Cleavage sequence[1)]
BrCN	M
BNPS-skatole	W
5-Nitro-5-thiocy-anobenzoate	C

[1)]Amino acid(s) in one-letter code.

[0091] Trypsin is preferably used, which specifically cleaves proteins and peptides at the C-terminal end of arginine. Such an enzyme is known, for example, from porcine or bovine pancreas or recombinant yeasts. Trypsin is particularly suitable for producing the desired polypeptides, if the lysine residues are protected and attacked by the enzyme.

[0092] The peptide sequence which can be cleaved by an endoproteinase is understood within the sense of the present invention as a short-chain peptide sequence which is preferably composed of 1 to 20 amino acids preferably 1 to 10 amino acids and contains a C-terminal cleavage site for the desired endoproteinase. This further peptide preferably additionally contains a combination of several amino acids (first part) between the N-terminal end and the desired endoproteinase recognition sequence, preferably selected from amino acids Gly, Thr, Ser, Ala, Pro, Asp, Glu, Arg and Lys. An amino acid stretch in which two to eight of these additional amino acids are the negatively charged amino acids Asp and/or Glu is preferably used as the first part.

[0093] Cleavage is also possible using BrCN (chemical cleavage) as long as the antifusogenic peptide does not contain methionine.

[0094] The fusion peptides are produced by expression of a DNA (nucleic acid sequence) which codes for the peptide in prokaryotes. The expression vector does not contain any elements that would mediate secretion of the fusion peptide into the medium or periplasm (e.g., signal peptides). Therefore, the peptide is formed as insoluble refractile bodies (IP's). DNA encoding the fusion peptide can be produced according to the methods known in the state of the art. It is further preferred to extend the nucleic acid sequence with additional regulation and transcription elements, in order to optimize the expression in the host cells. A DNA that is suitable for the expression can preferably be produced by synthesis. Such processes are familiar to a person skilled in the art and are described for example in Beattie, K. L., and Fowler, R. F., Nature 352 (1991) 548-549; EP-B 0 424 990; Itakura, K., et al., Science 198 (1977) 1056-1063. It may also be expedient to modify the nucleic acid sequence of the peptides according to the invention.

[0095] Such modifications are, for example:

[0096] modification of the nucleic acid sequence in order to introduce various recognition sequences of restriction enzymes to facilitate the steps of ligation, cloning and mutagenesis;

[0097] modification of the nucleic acid sequence to incorporate preferred codons for t he host cell;

[0098] extension of the nucleic acid sequence with additional regulation and transcription elements in order to optimize the expression in the host cell.

[0099] All further steps in the process for the production of suitable expression vectors and for the expression are state of the art and familiar to a person skilled in the art. Such methods are described for example in Sambrook et al., Molecular Cloning: A Laboratory Manual (1989), Cold Spring Harbor Laboratory Press, New York, USA.

[0100] As the fusion peptide is not secreted, it aggregates in the cell, preferably in the cytoplasm. Here the peptide is stored in a compressed and insoluble form (inclusion bodies). This reduces interference with cell functions like proteolytic degradation to a minimum. *Escherichia coli*, Streptomyces or Bacillus are for example suitable as prokaryotic host organisms. For the production of the fusion peptides according to the invention the prokaryotes are transformed in the usual manner with the vector which contains the DNA coding for the peptide and subsequently fermented in the usual manner. After lysis of the cells the insoluble inactive peptide (IBs) is isolated in the usual manner for example by centrifugation (pellet fraction). The desired insoluble peptide aggregates can if necessary be further enriched by washing the pellets e.g. with buffers containing detergents.

[0101] The insoluble fusion peptide is solubilized preferably with alkaline solutions (e.g., KOH, pH 10) and cleaved by appropriate means, e.g., factor Xa at pH 8.0.

[0102] Surprisingly it has turned out that the fusion peptides produced by the process according to the invention are not degraded in the host cells formed as inclusion bodies and, subsequently, can be completely cleaved enzymatically without significant cleavage in the antifusogenic peptide component itself.

[0103] The following examples, references, **FIG. 1** and the sequence listing are provided to aid the understanding of the present invention. It is understood that modifications can be made in the procedures set forth without departing from the spirit of the invention.

EXAMPLE 1

Expression Vector Construction

[0104] The synthetic gene Xa-T1357 has the following structure (from the N-terminus to the C-terminus):

[0105] EcoRI cleavage site

[0106] ribosome binding site of phage T5

[0107] amino acids M, R, G, S

[0108] amino acids 6xH (His-tag)

[0109] amino acids A, I, D, V

[0110] factor Xa linker I, E, G, R

[0111] T1357 sequence (39 amino acids)

[0112] amino acid G

[0113] 2 stop codons

[0114] BamHI cleavage site

[0115] and is described by SEQ ID NO:1.

[0116] The synthetic gene was constructed via gene synthesis with the use of four oligonucleotides consisting of the sequence of the gene with three overlapping regions of 20, 21 and 20 base pairs. Synthesis was performed by means of a two-step PCR reaction. In the first step, oligo 1 and 2 and oligo 3 and 4 each were applied as a template for complete synthesis of the N-terminal portion of the gene and the C-terminal portion of the gene, respectively. The products were used as a template for the second step, whereby equal portions of these products were applied. Primers 1 and 4 were used as the synthesis primers for this step.

[0117] The resulting synthetic gene was digested with EcoRI and BamHI, and so was the vector pQE-30 (Qiagen, DE). Both restriction digestions were purified by means of gel extraction (Qiagen) and then used for ligation for the production of an expression vector. The ratio of the insert to the vector was 3:1. The vector is shown in **FIG. 1**.

[0118] The sequence and the correct orientation of the construct were determined by means of restriction control and sequencing (SequiServe, DE).

EXAMPLE 2

Fermentation, IB Isolation and Solubilization

[0119] Fermentation and IB Isolation:

[0120] *E.coli* cells containing the expression vector of Example 1 are fermented in complex medium, with selection using ampicillin and kanamycin. LB medium is used for the pre-culture. For the main culture, yeast extract is used as the only complex component as the C- and N-source, and glycerine is used as an additional C-source. Both components are added in concentrated form during the fed-batch phase. Expression with IPTG is induced in a suitable growth phase. The pH value is adjusted to 7±0.2 using phosphoric acid/caustic soda. Fermentation is conducted with an overpressure of up to 0.5 bar. The oxygen partial pressure is controlled via the rotational speed of the stirrer, aeration and/or rate of dosage. After growth arrest and after a corresponding period of expression, the biomass is harvested, immediately or after cooling, by centrifugation and is stored in a frozen state.

[0121] The foreign protein to be expressed is obtained intracellularly in the form of inclusion bodies (IB), an IB preparation is conducted prior to purification. To this end, the biomass is disrupted with the use of a homogenizer and the IB's are purified in several washing and centrifugation steps.

[0122] Solubilization

[0123] 5.21 g of IB's (prepared from 20 g of cells by high-pressure disruption in 100 ml disruption buffer (100 mM Tris-HCl, 10 mM EDTA, pH 7.0)) are dissolved in 206 ml of 30 mM KOH pH 12 at room temperature, while stirring.

[0124] Protein content solubilisate: c=3.2 mg/ml (Bradford protein assay, Bradford M. M.,

[0125] Analytical Biochemistry 72 (1976) 248-254)→659.2 mg total protein.

[0126] Molecular weight of the fusion peptide of: MW=7161.96 (7156.05 monoisotopic)

[0127] The peptides

MRGS-HHHHHH-AIDV-IEGR-*RSV118*-G (SEQ ID NO:11)

MRGS-HHHHHH-AIDV-IEGR-*MV257*-G (SEQ ID NO:12)

[0128] are produced in the same manner, whereby the sequences RSV 118 and MV 257 (see Table 1) are used instead of the sequence "T 1357".

EXAMPLE 3

Citraconylation and Cleavage

[0129] a) Citraconylation

[0130] For citraconylation there is applied to the fusion peptide a mixture of citraconic acid anhydride (MW: 112.09, p=1.245 g/ml) and dioxane at a ratio of 1:1, whereby the citraconic acid anhydride is used at a 12-fold excess per mole of amino group. For citraconylation 0.66 g fusion peptide (MW: 7162, C=3.2 mg/ml in disruption buffer of Example 2b), five amino groups/molecule and 570 μl citraconic acid anhydride are required. The solubilisate is adjusted to a pH of 11 by using concentrated HCl. Subsequently, a mixture of 570 μl citraconic acid anhydride and 570 μl dioxane is added dropwise and the solution is buffered with 2 M KOH so that the pH value does not drop below 8.5. After adjusting the pH value to pH 10, the preparation is incubated overnight at room temperature, while stirring. The reaction is stopped with ethanol amine (21 ml of 1 M ethanol amine in H_2O, pH 8.0) (final concentration of ethanol amine in the solution: 100 mM) and the pH value is adjusted to pH 8.5 by using 2 M HCl. The citraconylated material is then stored at −20° C. Total volume of reaction batch: 237 ml.

[0131] b) Cleavage with Trypsin

[0132] The reaction batch is mixed with 11.85 ml of 1 M NaCl (final concentration 50 mM) and with 1.25 ml of 1 M $CaCl_2$ (final concentration 2 mM) and batches thereof of 10 ml each are incubated with 1.3 U/ml of trypsin for 80 minutes at room temperature, while stirring. At a level of trypsin of 1.3 U/ml, complete cleavage of the fusion protein has taken place. Cleavage is determined by means of 16%-tricin-SDS gels and HPLC analysis. The trypsin applied is trypsin from Roche Diagnostics GmbH (degree of purity II, 0.0091% chymotrypsin activity, activity: 206 U/ml).

[0133] c) Determination of Cleavage by HPLC Analysis

[0134] The portion of peptide is determined by HPLC with a linear calibration curve, which was prepared with synthetic Ac-T1357-G-OH. The following HPLC system is used:

[0135] Column: Pharmacia Source 15RPC ST 4.6/100

[0136] Eluent: buffer A: 20 mM Tris, pH 7.5; buffer B: 70% acetonitrile+30% buffer A

[0137] Gradient: in 28 minutes from 0% B to 100% B

[0138] Flow: 1 ml/min.

[0139] Detection: UV 226 nm

EXAMPLE 4

Purification

[0140] a) Ammonium Sulfate Precipitation

[0141] To the reaction mixture of example 3b solid ammonium sulfate (AS) (final concentration 2 M) is added and, after dissolving the AS, is stirred at room temperature. The precipitated peptide is removed by centrifugation (10,000 rpm) for 5 minutes at 4° C. and the pellet obtained (containing peptide and trypsin) is dissolved in 5 ml Tris buffer (50 mM, pH 8.5).

[0142] b) Benzamidine Sepharose 6B Chromatography

[0143] Removal of trypsin from the above-described solution is accomplished by means of a benzamidine column.

[0144] Application: 5 mg of protein

[0145] Column: HR 16 (Pharmacia, h=3.3 cm, d=1.6 cm, V=10 ml)

[0146] Material: Benzamidine Sepharose 6B (Pharmacia No. 17-0568-01), regenerated according to the manufacturer's instructions and equilibrated with 50 mM Tris buffer, pH 8.5

[0147] Flow: 1 ml/min.

[0148] Washing the column: 50 mM Tris buffer, pH. 8.5

[0149] 40 to 80% of the peptide flows through the column, the rest binds to the column material. In the subsequent elution with 60% of ethanol, a possibly present co-elution of the trypsin cannot be determined because trypsin is no longer detectable when the content of ethanol is 20% or higher. Both in the flow-through and in the elution, the peptide appears as a single peak in the HPLC analysis, and the peptide is also homogeneous in the SDS gel.

[0150] c) Purification via Phenyl Sepharose FF

[0151] Phenyl Sepharose FastFlow (Pharmacia): 5 mg of the pellet precipitate with AS (protein content c=5 mg/ml) are dissolved in 1 ml Tris buffer (50 mM Tris pH 8.5) and applied to the column. The peptide binds completely to the column material and can be eluted with an ethanol gradient of 20 to 60%, in 50 mM Tris HCl, pH 8.5 the elution taking place over a long period of retention. Peptide fractions are homogeneous.

SEQUENCE LISTING

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                                    1               5

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His His His Ala Ile Asp Val Ile Glu Gly Arg Trp Gln Glu Trp Glu
        10                  15                  20

cag aaa att acc gcc ctg ctg gaa cag gcg caa att cag caa gag aaa      148
Gln Lys Ile Thr Ala Leu Leu Glu Gln Ala Gln Ile Gln Gln Glu Lys
    25                  30                  35
```

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aac gaa tat gag ctg cag aaa ctg gat aag tgg gcg agc ctg tgg gaa      196
Asn Glu Tyr Glu Leu Gln Lys Leu Asp Lys Trp Ala Ser Leu Trp Glu
 40                  45                  50                  55

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Trp Phe Gly
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Gly Arg Trp Gln Glu Trp Glu Gln Lys Ile Thr Ala Leu Leu Glu Gln
             20                  25                  30

Ala Gln Ile Gln Gln Glu Lys Asn Glu Tyr Glu Leu Gln Lys Leu Asp
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Lys Trp Ala Ser Leu Trp Glu Trp Phe Gly
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1 5 10 15

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20 25 30

Ala Ser Leu Trp Glu Trp Phe
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Trp Asn Trp Phe
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RSV118

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Lys Ser Thr
35

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MV257

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20 25 30

Glu Leu Leu
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<210> SEQ ID NO 12
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<400> SEQUENCE: 12

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Gly Arg Leu His Arg Ile Asp Leu Gly Pro Pro Ile Ser Leu Glu Arg
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Leu Asp Val Gly Thr Asn Leu Gly Asn Ala Ile Ala Lys Leu Glu Asp
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Ala Lys Glu Leu Leu Gly
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M-HHHHHH-AIDV-IEGR-T1357-G

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Thr Ser Leu Ile His Ser Leu Ile Glu Glu Ser Gln Asn Gln Gln Glu
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-continued

Lys Asn Glu Gln Glu Leu Leu Glu Leu Asp Lys Trp Ala Ser Leu Trp
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Trp Phe Gly
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M-HHHHHH-IEGR-T680-G

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1 5 10 15

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Glu Leu Leu Glu Leu Asp Lys Trp Ala Ser Leu Trp Asn Trp Phe Gly
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MR-T1357-G

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sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:cleavage sequence

<400> SEQUENCE: 20

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1 5

What is claimed is:

1. A process for the production of an antifusogenic peptide by producing a fusion peptide of a length of about 14 to 70 amino acids in a prokaryotic host cell, comprising the steps, under such conditions that inclusion bodies of said fusion peptide are formed, of:

(a) expressing in said host cell a nucleic acid encoding said fusion peptide consisting of a first peptide which is an antifusogenic peptide of a length of about 10 to 50 amino acids and a second peptide of a length of about 4 to 30 amino acids, said first peptide being N-terminally linked to said second peptide;

(b) cultivating said host cell to produce said infusion bodies and;

(c) recovering said antifusogenic peptide from said inclusion bodies, wherein said recovered antifusogenic peptide consists of said fusion peptide or a peptide comprising the antifusogenic peptide of about 10 to 50 amino acids resulting from cleavage of said fusion peptide.

2. The process according to claim 1, wherein the second peptide consists of a peptide selected from:

(a) peptides which consist of from 4 amino acids to 20 amino acids,

(b) cleavable peptide linkers which consist of from 4 amino acids to 10 amino acids, and

(c) peptides consisting of

(i) a peptide consisting of up to 20 amino acids, and

(ii) a cleavable peptidic linker which consists of up to 10 amino acids, wherein the cleavable peptide linker is located between the N-terminus of the first peptide and the C-terminus of the peptide according to (i).

3. The process according to claim 1, wherein the ratio of the molecular weight of the first peptide to the molecular weight of the second peptide is from 10:1 to 1:2.

4. The process according to claim 1, further comprising recovering the inclusion bodies produced in accordance with

step (a) from said host cells, and solubilizing the recovered inclusion bodies, to recover said antifusogenic peptide from the inclusion bodies.

5. The process according to claim 4, wherein the first peptide is cleaved from the second peptide of the fusion peptide during or after solubilization of the recovered inclusion bodies.

6. A nucleic acid encoding a fusion peptide of from about 14 to 70 amino acids consisting of a first peptide which is an antifusogenic peptide selected from the group of SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, and said sequences further consisting of glycine at the C terminal end, N-terminally linked to a second peptide consisting of 4 to 30 amino acids.

7. The nucleic acid according to claim 6, wherein the second peptide is free from cysteine residues.

8. The nucleic acid according to claim 6, wherein the second peptide has an isoelectric point (IP) that differs from the IP of the first peptide by at least about 1 pH unit.

9. The nucleic acid according to claim 8, wherein the pH difference is from at least about 1 to 2 pH units.

10. The nucleic acid according to claim 6, wherein the second peptide comprises a polyhistidine group.

11. The nucleic acid according to claim 6, wherein the fusion peptide is selected from SEQ ID NO: 2, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14.

12. The nucleic acid according to claim 11, which consists of SEQ ID NO:1.

13. A prokaryotic expression vector comprising a nucleic acid according to claim 6.

14. A composition comprising inclusion bodies consisting essentially of at least one fusion peptide of from about 14 to 70 amino acids consisting of a first peptide which is an antifusogenic peptide selected from the group of SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, and said sequences further consisting of glycine at the C terminal end, N-terminally linked to a second peptide consisting of 4 to 30 amino acids, and optionally at least one peptide which is a fragment of said fusion peptide and which comprises the antifusogenic peptide of from about 10 to 50 amino acids.

15. A composition according to claim 14, wherein the fusion peptide is selected from SEQ ID NO: 2, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14.

* * * * *

10/716,095

11/19/02

What is claimed: CLMS 1-11; 1-5 RRS
6-11 WD → STRUCT?

112 P1 W.D. ART → DP178/T20

(1) A process for the recombinant production of an antifusogenic peptide by expression of a nucleic acid encoding the antifusogenic peptide as a repeat peptide in a microbial host cell to form inclusion bodies which comprise said repeat peptide, comprising the steps of washing the inclusion bodies with a denaturing agent at a pH value of at or below pH 6.5, solubilizing the washed inclusion bodies at a pH value of at least pH 9, and cleaving said repeat peptide to obtain said antifusogenic peptide.

WO02/103026

(2) The process according to claim 1, wherein the washing is performed from about pH 3 to about 5.

DP178
C34
DP107
T1249
T20
T1357

IFN-α2a SIC6-5 COPOX8 1957 - LINKER TRYPSIN CLEAVABLE PROTEIN LINKING

(3) The process according to claim 1, wherein said repeat peptide is cleaved during solubilization of said inclusion bodies.

(4) The process according to claim 1, wherein said repeat peptide is cleaved after solubilization of said inclusion bodies.

(5) The process according to claim 1, further comprising isolating the produced antifusogenic peptide.

US2003104581A1
6562787

6. A nucleic acid which encodes a fusion polypeptide consisting of (in N-terminal to C-terminal direction):

a) an antifusogenic peptide which is a repeat peptide of at least two identical antifusogenic peptide sequences; and

b) a peptide sequence which comprises a cleavage peptide and which is located between the antifusogenic peptide sequences.

WO99/59615

7. The nucleic acid according to claim 6, wherein the antifusogenic peptide sequence consists of from 10 to 100 amino acids.

5434067
6136564

8. The nucleic acid according to claim 6, wherein the repeat peptide consists of 2 to 20 identical antifusogenic peptide sequences.

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GUESS A-D 1996
I.M.
→ MATSUURA S 1999
LILLIE H 1998
HOCKETT RC 1994

9. The nucleic acid according to claim 6, wherein the antifusogenic peptide sequence is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, and fragments thereof.

10. The nucleic acid according to claim 6, wherein the peptide sequence which comprises a cleavage peptide is selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7.

11. A preparation of inclusion bodies comprising a fusion polypeptide, said fusion polypeptide comprising (in N-terminal to C-terminal direction):

a) an antifusogenic peptide which is a repeat peptide of at least 2 identical antifusogenic peptide sequences, each of which has a length of from about 10 to 100 amino acids; and

b) a cleavage peptide located between the antifusogenic peptidesequences.

the C-peptide of gp41. These antifusogenic peptides and fragments thereof are particularly useful in the invention.

Table 1

Name*	Name	Amino acid sequence (one-letter code)	SEQ ID NO:
T-1249	T1357[1]	WQEWEQKITALLEQAQIQQEKNEYELQKLDKWASLWEWF	1
T-20, DP178	T680[2]	YTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWF	2
T-118	RSV118[3]	FDASISQVNEKINQSLAFIRKSDELLHNVNAGKST	3
T-257	MV257[3]	LHRIDLGPPISLERLDVGTNLGNAIAKLEDAKELL	4

* for N-terminally acetylated and/or C-terminally amidated peptide; T-20 (synonymous with DP178) and T-1249 are from human immunodeficiency virus type 1 (HIV-1), T-118 is from respiratory syncytial virus (RSV) and T-257 is from measles virus (MV)

1) WO 99/59615

2) Rimsky, L.T., et al., J. Virol. 72 (1998) 986-993

3) Lambert, D.M., Proc. Natl. Acad. Sci. USA 93 (1996) 2186-2191

[0029] The length of the antifusogenic peptide is not critical. It is however preferred to use lengths of about 10 to 100 amino acids. The length must be sufficient to provide stability against denaturing agents at acidic pH values. The maximum length depends primarily on appropriate handling of the fusion polypeptides during solubilization, cleavage and purification.

[0030] In a preferred embodiment of the invention, the repeat peptide according to the invention is linked at its N-terminus with a further peptide of about 4 to 30 amino acids. The purpose of the further peptide is to impart additional properties to the antifusogenic peptide, for example, to improve expression (e.g. an N-terminal fragment of a polypeptide with a high expression rate such as interferon-α-2a, purification (e.g. a His-tag; see, e.g., Zhang, J.-H., et al., Nanjing Daxue Xuebao, Ziran Kexue 36(4) (2000) 515-517) or to allow subsequent N-terminal modification like acetylation or PEGylation.

such denaturing conditions and therefore can be removed by simple washing of the inclusion bodies under such denaturing conditions.

[0024] In a subsequent step, the inclusion bodies are solubilized at a pH value of 9 and higher for recovery of the soluble repeat peptide, whereby it is not necessary to add detergents or denaturing agents. After solubilisation, the repeat is cleaved chemically or enzymatically to obtain the desired antifusogenic peptide.

[0025] In a preferred embodiment of the invention, a cleavage sequence is located between the antifusogenic peptides of the repeat(s) and the repeat peptide is included in a fusion polypeptide characterized in that the repeats are linked together with said cleavage sequence(s) which are preferably of a length of about 1 to 10 amino acids. The antifusogenic peptide itself has a length of 10 to 100 amino acids, whereby (in regard to stable expression and formation of inclusion bodies) the length of the fusion polypeptide preferably is at least about 50 amino acids.

[0026] Therefore the invention provides an extremely simple method for recombinant production of antifusogenic peptides via the inclusion bodies route by simply washing the inclusion bodies and subsequently solubilizing them at different pH values.

[0027] "Antifusogenic" and "anti-membrane fusion" as used herein refer to a peptide's ability to inhibit or reduce the level of fusion events between two or more structures, e.g., cell membranes or viral envelopes or pili relative to the level of membrane fusion which occurs between the structures in the absence of the peptide. Examples hereof are peptidic inhibitors of lentiviruses such as human immunodeficiency virus (HIV), respiratory syncytical virus (RSV), human parainfluenza virus (HPV), measles virus (MEV), and Simian immunodeficiency virus (SIV). Such antifusogenic peptides are derived from C helix of a transmembrane subunit of an envelope fusion protein from a virus of the lentivirus genus and bind to the central coiled coil of the transmembrane subunit of the respective virus.

[0028] Especially preferred are HIV-1 antifusogenic peptides, preferably fragments of the C-peptide of gp41. Table 1 describes examples of HIV-1 antifusogenic peptides derived from

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                   to 50,000
 NEWS 22  DEC 01   CAS REGISTRY updated with new ambiguity codes
 NEWS 23  DEC 11   CAS REGISTRY chemical nomenclature enhanced
 NEWS 24  DEC 14   WPIDS/WPINDEX/WPIX manual codes updated
 NEWS 25  DEC 14   GBFULL and FRFULL enhanced with IPC 8 features and
                   functionality
 NEWS 26  DEC 18   CA/CAplus pre-1967 chemical substance index entries enhanced
                   with preparation role
 NEWS 27  DEC 18   CA/CAplus patent kind codes updated
 NEWS 28  DEC 18   MARPAT to CA/CAplus accession number crossover limit increased
                   to 50,000
 NEWS 29  DEC 18   MEDLINE updated in preparation for 2007 reload
 NEWS 30  DEC 27   CA/CAplus enhanced with more pre-1907 records

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               MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
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=> d l1,cbib,clm

L1 ANSWER 1 OF 1 USPATFULL on STN
2005:220994 Methods for the recombinant production of antifusogenic peptides.
Kaczmarek, Alexandra, Penzberg, GERMANY, FEDERAL REPUBLIC OF
Kopetzki, Erhard, Penzberg, GERMANY, FEDERAL REPUBLIC OF
Schantz, Christian, Thalhausen, GERMANY, FEDERAL REPUBLIC OF
Seeber, Stefan, Penzberg, GERMANY, FEDERAL REPUBLIC OF
US 2005191729 A1 20050901
APPLICATION: US 2003-716095 A1 20031118 (10)
PRIORITY: EP 2003-988 20030117
EP 2002-25618 20021119
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A process for the recombinant production of an antifusogenic peptide by expression of a nucleic acid encoding the antifusogenic peptide as a repeat peptide in a microbial host cell to form inclusion bodies which comprise said repeat peptide, comprising the steps of washing the inclusion bodies with a denaturing agent at a pH value of at or below pH 6.5, solubilizing the washed inclusion bodies at a pH value of at least pH 9, and cleaving said repeat peptide to obtain said antifusogenic peptide.

2. The process according to claim 1, wherein the washing is performed from about pH 3 to about 5.

3. The process according to claim 1, wherein said repeat peptide is cleaved during solubilization of said inclusion bodies.

4. The process according to claim 1, wherein said repeat peptide is cleaved after solubilization of said inclusion bodies.

5. The process according to claim 1, further comprising isolating the produced antifusogenic peptide.

6. A nucleic acid which encodes a fusion polypeptide consisting of (in N-terminal to C-terminal direction): a) an antifusogenic peptide which is a repeat peptide of at least two identical antifusogenic peptide sequences; and b) a peptide sequence which comprises a cleavage peptide and which is located between the antifusogenic peptide sequences.

7. The nucleic acid according to claim 6, wherein the antifusogenic peptide sequence consists of from 10 to 100 amino acids.

8. The nucleic acid according to claim 6, wherein the repeat peptide consists of 2 to 20 identical antifusogenic peptide sequences.

9. The nucleic acid according to claim 6, wherein the antifusogenic peptide sequence is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, and fragments thereof.

10. The nucleic acid according to claim 6, wherein the peptide sequence which comprises a cleavage peptide is selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7.

11. A preparation of inclusion bodies comprising a fusion polypeptide, said fusion polypeptide comprising (in N-terminal to C-terminal direction): a) an antifusogenic peptide which is a repeat peptide of at least 2 identical antifusogenic peptide sequences, each of which has a length of from about 10 to 100 amino acids; and b) a cleavage peptide located between the antifusogenic peptidesequences.

```
=> e kopetzki erhard/in
E1            1     KOPETZ KURT/IN
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E6            1     KOPETZKY CHRISTOPHER A/IN
E7            3     KOPETZKY HORST/IN
E8            1     KOPETZKY MARKUS/IN
E9            2     KOPETZKY PETER/IN
E10          32     KOPETZKY ROBERT/IN
E11           1     KOPETZKY ROBERT THOMAS/IN
E12           1     KOPEZEWSKI MICHAEL T/IN

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          6395 INCLUSION BODIES
                 (INCLUSION(W)BODIES)
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                 ((INCLUSION(W)BODIES)/CLM)
L4            3 L3 AND (INCLUSION BODIES/CLM)

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L5            2 L4 NOT L1

=> d l5,cbib,clm,1-2

L5   ANSWER 1 OF 2  USPATFULL on STN
2005:4382 Method for recombinant production of polypeptides.
    Gruenbeck, Rainer, Penzberg, GERMANY, FEDERAL REPUBLIC OF
    Kopetzki, Erhard, Penzberg, GERMANY, FEDERAL REPUBLIC OF
    Popp, Friedrich, Penzberg, GERMANY, FEDERAL REPUBLIC OF
    US 2005003485 A1 20050106
    APPLICATION: US 2004-866567  A1  20040612 (10)
    PRIORITY: EP 2003-12295  20030612
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM    What is claimed is:
       1. An Improved method for the recombinant production of a polypeptide by
       expressing a nucleic acid encoding said polypeptide in a microbial host
       cell, forming in the cytoplasm of said host cell inclusion bodies
       containing said polypeptide, and isolating, solubilizing and naturing
       said polypeptide, wherein the improvement comprises incubating the host
       cell or the host cell content following fermentation at a temperature of
       40° C. or higher for at least 10 minutes and subsequently
       isolating insoluble polypeptide from the host cell.

       2. The method according to claim 1, characterized in that the
       fermentation is performed at a temperature of 30° C. or lower.
```

3. The method according to claim 1 or 2, characterized in that the incubation is carried out for 10 to 180 minutes.

4. The method according to claims 1 to 3, characterized in that the incubation is performed at a temperature of 40° C. to 60° C.

L5 ANSWER 2 OF 2 USPATFULL on STN
2003:294387 Autocatalytically activatable zymogenic precursors of proteases and their use.
Kopetzki, Erhard, Penzberg, GERMANY, FEDERAL REPUBLIC OF
Hopfner, Karl-Peter, Maierhoefen, GERMANY, FEDERAL REPUBLIC OF
Bode, Wolfram, Gauting, GERMANY, FEDERAL REPUBLIC OF
Huber, Robert, Gernering, GERMANY, FEDERAL REPUBLIC OF
US 2003207402 A1 20031106
APPLICATION: US 2003-446065 A1 20030527 (10)
PRIORITY: EP 1997-114513 19970822
EP 1997-117816 19971015
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A process for the recombinant production of a serine protease comprising: (a) transforming a prokaryotic host cell with a recombinant nucleic acid coding for a zymogenic precursor of said protease, wherein said precursor is characterized by having a naturally occurring, non-autocatalytic cleavage site replaced by an autocatalytic cleavage site which is recognized by said protease, whereby said precursor is cleaved at said site by said protease to produce said protease, (b) culturing said host cell such that said precursor is formed in said cell in the form of **inclusion bodies**, (c) isolating said **inclusion bodies** containing said precursor, (d) renaturing said precursor, and (e) cleaving said precursor autocatalytically to produce said protease.

2. The process of claim 1, wherein said protease is selected from the group consisting of trypsin, thrombin, factor Xa and lysyl endoproteinase.

3. The process of claim 1, wherein said zymogenic precursor is characterized by having a ratio of proteolytic activity to that of said active serine protease of 1:5 or less.

4. An autocatalytically cleavable zymogenic precursor of a serine protease characterized by having a naturally occuring cleavage site replaced by an autocatalytic cleavage site which does not occur naturally.

5. A process for the recombinant production of an autocatalytically cleavable zymogenic precursor of a serine protease which contains no autocatalytic cleavage site in its naturally occurring form, said process comprising: (a) transforming a host cell with a recombinant nucleic acid coding for said precursor, (b) culturing said host cell and expressing said nucleic acid such that said precursor is formed in said cell in the form of **inclusion bodies**, (c) isolating said **inclusion bodies**.

6. A process for the recombinant production of **inclusion bodies** which contain an autocatalytically cleavable zymogenic precursor of a serine protease, said protease characterized by containing no autocatalytic cleavage site in its naturally occurring form, said process comprising: (a) transforming a host cell with a recombinant nucleic acid coding for said precursor, (b) culturing said host cell and expressing said nucleic acid such that said precursor is formed in said cell in the form of **inclusion bodies**, (c) isolating said **inclusion bodies** containing said precursor.

7. A recombinant, autocatalytically cleavable precursor of a serine protease which contains no autocatalytic cleavage site in its naturally occurring form, said precursor prepared by the process comprising: (a) transforming a host cell with a recombinant nucleic acid coding for said precursor, (b) culturing said host cell and expressing said nucleic acid such that said precursor is formed in said cell in the form of **inclusion bodies**, (c) isolating said **inclusion bodies**.

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=> e schantz christian/iin
'IIN' IS NOT A VALID EXPAND FIELD CODE FOR FILE 'USPATFULL'
The indicated field code is not available for EXPAND in this
file.  To see a list of valid EXPAND field codes, enter HELP
SFIELDS at an arrow prompt (=>).

=> d his

     (FILE 'HOME' ENTERED AT 11:43:32 ON 08 JAN 2007)

     FILE 'USPATFULL' ENTERED AT 11:43:44 ON 08 JAN 2007
               E KACZMAREK ALEXANDRA/IN
L1              1 S E3
               E KOPETZKI ERHARD/IN
L2             26 S E3
L3             16 S L2 AND (INCLUSION BODIES)
L4              3 S L3 AND (INCLUSION BODIES/CLM)
L5              2 S L4 NOT L1

=> s l3 not l4
L6             13 L3 NOT L4

=> s l6 and ay<2003
     3803600 AY<2003
L7             13 L6 AND AY<2003

=> d l7,cbib,clm;1-13

L7   ANSWER 1 OF 13  USPATFULL on STN
2003:321425 Chimeric serine proteases.
    Bode, Wolfram, Gauting, GERMANY, FEDERAL REPUBLIC OF
    Engh, Richard, Wessling, GERMANY, FEDERAL REPUBLIC OF
    Hopfner, Karl-Peter, Munich, GERMANY, FEDERAL REPUBLIC OF
    Huber, Robert, Germering, GERMANY, FEDERAL REPUBLIC OF
    Kopetzki, Erhard, Penzberg, GERMANY, FEDERAL REPUBLIC OF
    Boehringer Mannheim GmbH, Mannheim, GERMANY, FEDERAL REPUBLIC OF (non-U.S.
    corporation)
    US 6660492 B1 20031209
    APPLICATION: US 2000-664595  20000918 (9)
    PRIORITY: EP 1997-121232  19971203
    DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM    What is claimed is:
       1. A method for determining whether a substance has serine protease
       inhibiting activity, comprising: a) combining a chimeric protein with a
       target substance under conditions such that the chimeric protein
       exhibits serine protease activity in the absence of the target
       substance, wherein the chimeric protein comprises a first sequence and a
       second sequence C-terminal to the first sequence and linked to the first
       sequence by one or more peptide bonds, the first sequence having the
       amino acid sequence of the first catalytic domain half of a first serine
       protease and the second sequence having the amino acid sequence of the
       second catalytic domain half of a second serine protease different from
       the first serine protease; wherein the first sequence has the amino acid
       sequence of the first catalytic domain half of factor Xa and the second
       sequence has the amino acid sequence of the second catalytic domain half
       of trypsin; b) measuring the serine protease activity of the chimeric
       protein in the presence of the target substance and in the absence of
       the target substance, to detect whether the serine protease activity of
       the chimeric protein in the presence of the target substance is
       decreased relative to the serine protease activity of the chimeric
       protein in the absence of the target substance; and c) if a decrease in
       serine protease activity is detected, determining that the target
       substance has serine protease inhibiting activity.

       2. The method according to claim 1, wherein the chimeric protein
       comprises the amino acid sequence encoded by SEQ ID NO:12.

       3. The method according to claim 2, wherein the chimeric protein
       consists of the amino acid sequence of SEQ ID NO: 15.

       4. The method according to claim 1, wherein the chimeric protein is in
       crystalline form.
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5. The method according to claim 1, wherein the chimeric protein comprises the amino acid sequence of SEQ ID NO: 15 having a mutation selected from the group consisting of amino acid mutations shown in FIG. 2 and combinations thereof.

L7 ANSWER 2 OF 13 USPATFULL on STN
2002:116011 Recombinant inactive avidin mutants.
Kopetzki, Erhard, Penzberg, GERMANY, FEDERAL REPUBLIC OF
Muller, Rainer, Penzberg, GERMANY, FEDERAL REPUBLIC OF
Engh, Richard, Munchen, GERMANY, FEDERAL REPUBLIC OF
Schmitt, Urban, Oberhausen, GERMANY, FEDERAL REPUBLIC OF
Deger, Arno, Pernzberg, GERMANY, FEDERAL REPUBLIC OF
Brandstetter, Hans, Chelsea, GERMANY, FEDERAL REPUBLIC OF
Roche Diagnostics GmbH, Mannheim, GERMANY, FEDERAL REPUBLIC OF (non-U.S. corporation)
US 6391571 B1 20020521
APPLICATION: US 1999-366862 19990804 (9)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. An isolated polypeptide which is capable of binding to biotin and has an affinity therefore of less than 10^{10} l/mol, which is a mutein of avidin which differs in its amino acid sequence by at least one amino acid as compared to avidin.

2. The polypeptide of claim 1, wherein said mutein of avidin differs from avidin in at least two amino acids.

3. The polypeptide of claim 1, wherein said mutein of avidin differs from avidin in that at least 3 amino acids are substituted.

4. An isolated polypeptide which is a mutein of avidin which has the amino acid sequence set out at SEQ ID NO: 3, wherein said mutein has an amino acid sequence which differs from the amino acid sequence of avidin set forth in SEQ ID NO: 3 by having a different amino acid at at least one position selected from the group consisting of Leu 14, Ser 16, Tyr 33, Thr 35, Val 37, Thr 38, Ser 75, Thr 77, Leu 99, and Ile 117, and which is capable of binding to biotin with an affinity less than 10^{10} l/mol.

5. The polypeptide of claim 4, wherein said mutein has a different amino acid at at least one position selected from the group consisting of Leu 14, Ser 16, Thr 35, and Leu 99.

6. The polypeptide of claim 5, wherein said at least one position is replaced by Arg, Trp, Tyr, Phe, or His.

7. The polypeptide of claim 4, wherein said mutein differs from avidin in at least two amino acid positions.

8. The polypeptide of claim 7, wherein said at least two amino acid positions are substituted by Arg, Trp, Tyr, Phe or His.

9. The polypeptide of claim 2, wherein said mutein of avidin differs from avidin as set forth in SEQ ID NO: 3 in at least two positions, selected from the group consisting of Leu 14, Ser 16, Thr 35 and Leu 99.

10. The polypeptide of claim 9, wherein said at least two positions are substituted by Arg, Trp, Tyr, Phe or His.

11. The polypeptide of claim 3, wherein said mutein of avidin differs from avidin as set forth in SEQ ID NO: 3 in at least three positions, selected from the group consisting of Leu 14, Ser 16, Thr 35 and Leu 99.

12. The polypeptide of claim 11, wherein said positions are substituted by Arg, Trp, Tyr, Phe or His.

13. A polymeric conjugate comprising the polypeptide of claim 1 and a second molecule conjugated thereto.

14. A polymeric conjugate comprising the polypeptide of claim 4 and a second molecule conjugated thereto.

15. A polymeric conjugate comprising the polypeptide of claim 11 and a second molecule conjugate thereto.

16. The polymeric conjugate of claim 13, 14 or 15, wherein said second molecule is a polypeptide or a protein.

17. The polymeric conjugate of claim 16, wherein said second molecule is bovine serum albumin.

18. The polypeptide of claim 1 or 4 wherein said polypeptide is capable of forming a dimer or a tetramer.

19. The polypeptide of claim 11, wherein said polypeptide is capable of forming a dimer or a tetramer.

20. Interference elimination reagent comprising the polypeptide of claim 1, 4, or 11 immobilized to a solid phase.

21. The interference elimination reagent of claim 20, wherein said solid phase is a chip, a membrane, a microtitre plate, a reaction vessel, or a microbead.

22. A method for determining an analyte in a sample, comprising adding an analyte detection system to said sample, said sample comprising biotin and avidin, and the polypeptide of claim 1, 4, or 11 in an amount sufficient to reduce any interference with binding of said biotin and avidin and determining said analyte.

23. Composition comprising an isolated mutein of avidin which has affinity for biotin of from about 10^5 to about 10^{11} l/mol, wherein said mutein differs from avidin by at least one amino acid, coated onto a solid phase.

24. The polypeptide of claim 1, wherein said mutein of avidin differs from avidin in that amino acids Leu 14, Ser 16, Thr 35 and Leu 99 are substituted.

L7 ANSWER 3 OF 13 USPATFULL on STN
2001:196819 Recombinant inactive core streptavidin mutants.
Kopetzki, Erhard, Penzberg, Germany, Federal Republic of
Muller, Rainer, Penzberg, Germany, Federal Republic of
Engh, Richard, Munchen, Germany, Federal Republic of
Schmitt, Urban, Oberhausen, Germany, Federal Republic of
Deger, Arno, Pernzberg, Germany, Federal Republic of
Brandstetter, Hans, Chelsea, MA, United States
Roche Diagnostics GmbH, Germany, Federal Republic of (non-U.S. corporation)
US 6312916 B1 20011106
APPLICATION: US 1997-831399 19970401 (8)
PRIORITY: DE 1996-19613053 19960401
DE 1996-19637718 19960916
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A polypeptide which is a mutein of wild type streptavidin which has the amino acid sequence set out at SEQ ID NO: 1, wherein said mutein has an amino acid sequence which differs from the amino acid sequence of wild -type streptavidin as set forth in SEQ ID NO: 1 by having a different amino acid at at least one position selected from the group consisting of Leu 25, Ser 27, Tyr 43, Ser 45, Val 47, Gly 48, Ser 88, Thr 90, Leu 10, and Asp 128, wherein said mutein has reduced affinity for biotin as compared to the affinity for biotin had by wild-type streptavidin, the amino acid sequence of which is set forth at SEO ID NO: 1.

2. The polypeptide of claim 1, wherein said mutein of streptavidin differs from streptavidin by having a different amino acid at at least one position selected from the group consisting of Leu 25, Ser 27, Ser 45, and Leu 110.

3. The polypeptide of claim 2, wherein said mutein of streptavidin is substituted at Leu 25, Ser 27, Ser 45 or Leu 110 by Arg, Trp, Tyr, Phe or His.

4. The polypeptide of claim 1, wherein said mutein of streptavidin

differs from streptavidin in at least two amino acids.

5. The polypeptide of claim 4, wherein said mutein of streptavidin differs in at least two positions which are selected from the group consisting of Leu 25, Ser 27, Ser 45, and Leu 110.

6. The polypeptide of claim 5, wherein said mutein is substituted at said at least two positions by Arg, Trp, Tyr, Phe, or His.

7. The polypeptide of claim 1, wherein said mutein of streptavidin differs from streptavidin in that at least 3 amino acids are substituted.

8. The polypeptide of claim 7, wherein said mutein of streptavidin differs from streptavidin in at least three positions, selected from the group consisting of Leu 25, Ser 27, Ser 45, and Leu 110.

9. The polypeptide of claim 8, wherein said mutein is substituted at said at least three positions by Arg, Trp, Tyr, Phe or His.

10. A polymeric conjugate comprising the polypeptide of claim 1 and a second molecule conjugated thereto.

11. The polymeric conjugate of claim 10 wherein said second molecule is a polypeptide or a protein.

12. The polymeric conjugate of claim 11, wherein said second molecule is bovine serum albumin.

13. The polypeptide of claim 1, wherein said polypeptide is capable of forming a dimer or a tetramer.

14. Interference elimination reagent comprising the polypeptide of claim 1, immobilized to a solid phase.

15. The interference elimination reagent of claim 14, wherein said solid phase is a chip, a membrane, a microtitre plate, a reaction vessel, or a microbead.

16. A method for determining an analyte in a sample, comprising adding an analyte detection system to said sample, said sample comprising biotin and either avidin or streptavidin, and the polypeptide of claim 1, in an amount sufficient to reduce any interference with binding of said biotin and avidin or streptavidin and determining said analyte.

17. Composition comprising a mutein of wild type streptavidin the amino acid sequence of which is set forth at SEQ ID NO: 1 which has an affinity for biotin of from about 10^5 to about 10^{11} 1/mol, wherein said mutein differs from streptavidin by at least one amino acid selected from the group consisting of Leu 25, Ser 27, Tyr 43, Ser 45, Val 47, Gly 48, Ser 88, Thr 90, Leu 110, and Asp 148, coated on to a solid phase.

18. The polypeptide of claim 1, which has an affinity for biotin of less than about 10^{10} 1/mole.

19. A polypeptide which is a mutein of wild type streptavidin which has the amino acid sequence set out at SEQ ID NO: 1, wherein said mutein has an amino acid sequence which differs from the amino acid sequence of wild-type streptavidin as set forth in SEQ ID NO: 1 by having a different amino acid at at least one position selected from the group consisting of Leu 25, Ser 27, Tyr 43, Ser 45, Val 47, Gly 48, Ser 88, Thr 90, Leu 110, and Asp 128, wherein said mutein has reduced affinity for biotin as compared to the affinity for biotin had by wild-type streptavidin, the amino acid sequence of which is set forth at SEQ ID NO: 1 and has the ability to dimerize or tetramerize.

20. The polypeptide of claim 18, wherein said mutein of streptavidin differs from streptavidin by having a different amino acid at at least one position selected from the group consisting of Leu 25, Ser 27, Ser 45, and Leu 110.

21. The polypeptide of claim 20, wherein said mutein of streptavidin is substituted at Leu 25, Ser 27, Ser 45 or Leu 110 by Arg, Trp, Tyr, Phe or His.

22. The polypeptide of claim 19, wherein said mutein of streptavidin or said mutein of avidin differs from streptavidin or avidin in at least two amino acids.

23. The polypeptide of claim 22, wherein said mutein of streptavidin differs in at least two positions which are selected from the group consisting of Leu 25, Ser 27, Ser 45, and Leu 110.

L7 ANSWER 4 OF 13 USPATFULL on STN
2001:158077 Host-vector system.
Kopetzki, Erhard, Penzberg, Germany, Federal Republic of
Schantz, Christian, Thalhausen, Germany, Federal Republic of
Roche Diagnostics GmbH, Mannheim, Germany, Federal Republic of (non-U.S. corporation)
US 6291245 B1 20010918
APPLICATION: US 1999-344888 19990625 (9)
PRIORITY: EP 1998-113156 19980715
EP 1998-119078 19981009
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A prokaryotic expression vector for use in a host cell which comprises: a) a prokaryotic origin of replication; b) at least one eukaryotic auxotrophy marker gene which encodes an enzyme required for the sythesis of a product necessary for the survival of an auxotrophic prokaryote under the control of a eukaryotic promoter; c) a foreign gene under the direct control of a prokaryotic promoter, such that the mRNA of the foreign gene is formed; and d) one or more transcription terminators; which prokaryotic expression vector contains no more than fifty consecutive base pairs of a host cell sequence.

2. The prokaryotic expression vector of claim 1, which vector further comprises a ribosomal binding site and a multiple cloning site.

3. The prokaryotic expression vector of claim 1 wherein the auxotrophy marker gene and the foreign gene are oriented in opposite directions with the transcription terminator located between the auxotrophy marker gene and the foreign gene so positioned as to terminate transcription of both genes.

4. The prokaryotic expression vector of claim 1 wherein the auxotrophy marker gene is a yeast gene.

5. The prokaryotic expression vector of claim 3 wherein the auxotrophy marker gene is a yeast gene.

6. The prokaryotic expression vector of claim 5 wherein the auxotrophy marker gene is URA3 or TRP 1.

7. The prokaryotic expression vector of claim 6 wherein the auxotrophic marker gene is expressed from the 5' flanking region of the URA3 or TRP1 gene.

8. The prokaryotic expression vector of claim 7 wherein the prokaryotic promoter is T5-P N25/03/04.

9. The prokaryotic expression vector of claim 8 wherein the transcription terminator is derived from bacteriophage fd or λ-T0.

10. The prokaryotic expression vector of claim 9 wherein the origin of replication is derived from pBR or pUC plasmids.

11. A prokaryotic auxotrophic host cell comprising the prokaryotic expression vector of claim 1, which host cell has at least one mutation such that it is unable to express the product expressed by the auxotrophic marker gene.

12. The prokaryotic host cell of claim 11 which is an E. coli cell.

13. The prokaryotic host cell of claim 12 which is unable to produce the product of one of the trpC gene or the pyrF gene.

14. The prokaryotic host cell of claim 13 which comprises the prokaryotic expression vector of claim 1 wherein the auxotrophy marker gene is URA3 or TRP1, and wherein the auxotrophy marker gene and the foreign gene are oriented in opposite directions, with the transcription terminator located between the auxotrophy marker gene and the foreign gene, so positioned as to terminate transcription of both genes.

15. The prokaryotic host cell of claim 14 which produces sufficient amounts of the product of the genes URA3 or TRP1 to survive on minimal medium but does not produce an amount of such gene product which constitutes more than 1.0% of the total amount of protein produced by the cell.

16. A method for producing a recombinant protein which comprises expressing a gene encoding said recombinant protein under the direct control of a prokaryotic promoter in a prokaryotic expression vector, which prokaryotic expression vector contains no more than fifty consecutive base pairs of a host cell sequence, wherein said prokaryotic expression vector additionally comprises a prokaryotic origin of replication; at least one eukaryotic auxotrophy marker gene which encodes an enzyme required for the synthesis of a product necessary for the survival of an auxotrophic prokaryote under the control of a eukaryotic promoter; and one or more transcription terminators.

L7 ANSWER 5 OF 13 USPATFULL on STN
2001:147695 Process for the production of proteins in soluble form by modulation of an inducible promoter.
Kopetzki, Erhard, Penzberg, Germany, Federal Republic of
Schumacher, Gunther, Bernried, Germany, Federal Republic of
Roche Diagnostics GmbH, Mannheim, Germany, Federal Republic of (non-U.S. corporation)
US 6284484 B1 20010904
APPLICATION: US 1991-725943 19910627 (7)
PRIORITY: DE 1987-3723992 19870720
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A process for the expression of a protein in a transformed Escherichia coli host cell containing a DNA sequence encoding said protein and controlled by an inducible promoter, said process comprising limiting induction of said promoter to less than 10% of the maximum induction of said promoter thereby producing a greater amount of soluble and active forms of said protein than in the absence of the limited induction of said promoter.

2. The process of claim 1, comprising limiting induction of said promoter to less than 5% of the maximum induction.

3. The process of claim 1, comprising limiting induction of said promoter to less than 1% of the maximum induction.

4. The process of claim 1, wherein said host cell is an Escherichia coli cell containing a lac-Iq gene.

5. The process of claim 1, wherein said host cell is an Escherichia coli DSM 2102 cell.

6. The process of claim 1, comprising limiting induction during the logarithmic growth phase of said host cell.

7. The process of claim 4, wherein said promoter is a naturally occurring promoter.

8. The process of claim 7, wherein said promoter is a lac, lacuv 5, trp, tac, trc, rac, phoA, mgl, λ-P_L, λ-P_R, T5, T7, or SP6 promoter.

9. The process of claim 1, comprising limiting induction by restricting addition of inducer.

10. The process of claim 8, wherein said promoter is a lac promoter or a derivative of a lac promoter.

11. The process of claim 1, wherein said promoter is a lac promoter or a

derivative of a lac promoter and said limiting induction comprises adding isopropyl-β-D-thiogalactopyranoside in a concentration less than 0.01 mM.

12. The process of claim 1, wherein said promoter is a lac promoter and said limiting induction comprises adding lactose at a concentration of less than 1%.

13. The process of claim 1, wherein said host cell is an Escherichia coli ED82-Iq cell.

14. The process of claim 1, wherein said host cell is a microorganism containing a lac promoter and a lac repressor mutant gene lacI^s.

15. The process of claim 1, wherein said promoter has a catabolite activator protein site and said promoter has reduced affinity for the catabolite activator protein.

16. The process according to claim 1, wherein the maximum induction of said promoter is determined by comparison with a standard system of said transformed Escherichia coli host cell, wherein said standard system consists of the expression of beta-galactosidase in said host cell under the control of the same inducible promoter using an inducer concentration of 0.1 to 1 mmol/l IPTG.

17. The process of claim 16, comprising limiting induction of said promoter to less than 5% of the maximum induction.

18. The process of claim 16, comprising limiting induction of said promoter to less than 1% of the maximum induction.

19. The process of claim 16, wherein said host cell is an Escherichia coli cell containing a lac-Iq gene.

20. The process of claim 16, wherein said host cell is an Escherichia coli DSM 2102 cell.

21. The process of claim 16, comprising limiting induction during the logarithmic growth phase of said host cell.

22. The process of claim 19, wherein said promoter is a naturally occurring promoter.

23. The process of claim 22, wherein said promoter is a lac, lacuv 5, trp, tac, trc, rac, phoA, mgl, λ-P_l, λ-P_r, T5, T7, or SP6 promoter.

24. The process of claim 16, comprising limiting induction by restricting addition of inducer.

25. The process of claim 23, wherein said promoter is a lac promoter or a derivative of a lac promoter.

26. The process of claim 16, wherein said promoter is a lac promoter or a derivative of a lac promoter and said limiting induction comprises adding isopropyl-B-D-thiogalactopyranoside in a concentration less than 0.01 mM.

27. The process of claim 16, wherein said promoter is a la promoter and said limiting induction comprises adding lactose at a concentration of less than 1%.

28. The process of claim 16, wherein said host cell is an Escherichia coli ED82-Iq cell.

29. The process of claim 16, wherein said host cell is a microorganism containing a lac promoter and a lac repressor mutant gene lacI^s.

30. The process of claim 16, wherein said promoter has a catabolite activator protein site and said promoter has reduced affinity for the catabolite activator protein.

L7 ANSWER 6 OF 13 USPATFULL on STN
2001:136423 Recombinant blood-coagulation proteases.

Kopetzki, Erhard, Deutschland, Germany, Federal Republic of
Hopfner, Karl-Peter, Deutschland, Germany, Federal Republic of
Roche Diagnostics GmbH, Mannheim, Germany, Federal Republic of (non-U.S. corporation)
US 6277618 B1 20010821
WO 9747737 19971218
APPLICATION: US 1999-202101 19990412 (9)
WO 1997-EP3027 19970611 19990412 PCT 371 date 19990412 PCT 102(e) date
PRIORITY: DE 1996-110959 19960606
DE 1996-109288 19960611
DE 1996-110109 19960622
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. An isolated, non-glycosylated, enzymatically active protein with serine protease activity or a zymogenic form thereof consisting of the following domains of a member of the factor IX family: (a) at least one of either an EGF1 or EGF2 domain, linked by its C-teiminus to, (b) the N-teiminus of a zymogen activation domain, which is linked by its C-teirminus to, (c) the N-teiminus of a catalytic domain.

2. The isolated protein of claim 1, wherein (a) is an EGF1 domain.

3. The isolated protein of claim 1, wherein (a) is an EGF2 domain.

4. The isolated protein of claim 1, wherein (a) consists of an EGF 1 domain linked by its C-terminus to the N-teiminus of an EGF2 domain.

5. The isolated protein of claim 1 wherein at least one of (a), (b), and (c), is from a member of the factor IX family which differs from the family from which the other domains are from.

6. The isolated protein of claim 1, wherein each of (a), (b), and (c) is from a different member of the factor IX family.

7. The isolated protein of claim 1, wherein said EGF2 domain and said catalytic domain are from factor X, and said zymogen activation domain is from factor IX.

8. The isolated protein of claim 1 wherein (a), (b), and (c), are from either factor IX or factor X.

9. The isolated protein of claim 8, wherein at least one of (a), (b), and (c) is from factor IX, and the remaining domains are from factor X.

10. The isolated protein of claim 8, wherein at least one of (a), (b), and (c) is from factor X, and the remaining domains are from factor IX.

11. A process for the manufacture of the protein of claim 1, comprising transforming a prokaryotic cell with an expression vector that encodes said protein and culturing the transfoimed prokaiyotic cell under conditions favoring production of said protein.

12. A method for determining if a substance is an inhibitor or activator of a member of the factor IX family, comprising contacting said substance with the isolated protein of claim 1, and determining activity of said protein, wherein a change in said activity, relative to said activity prior to said contact is indicative of activator or inhibitor properties of said substance.

13. A method for the determination of factor IXa in a sample comprising incubating a zymogenic form of the protein of claim 1 with a substrate cleavable by an active form of said zymogen, wherein determining cleavage of said substrate is indicative of factor IXa in a sample.

14. The method of claim 13, wherein said sample is a body fluid.

15. The method of claim 13, wherein said cleavable substrate is a chromogenic substrate.

16. The method of claim 13, wherein the cleavable substrate is a second zymogenic form of an enzyme activatable by the active form of the protein of claim 1, which is activatable by the factor IXa.

17. An isolated, non-glycosylated, enzymatically active protein with

serine protease activity or a zymogenic form thereof, consisting of an amino acid sequence encoded by nucleotides 322 to 462 of SEQ ID NO: 15, concatenated to an amino acid sequence encoded by nucleotides 535 to 1005 of the nucleotide sequence set forth in SEQ ID NO: 16, concatenated to an amino acid sequence encoded by nucleotides 964 to 1362 as set forth in SEQ ID NO: 15.

L7 ANSWER 7 OF 13 USPATFULL on STN
2001:4516 Chimeric serine proteases.
Bode, Wolfram, Gauting, Germany, Federal Republic of
Engh, Richard, Wessling, Germany, Federal Republic of
Hopfner, Karl-Peter, Munich, Germany, Federal Republic of
Huber, Robert, Germering, Germany, Federal Republic of
Kopetzki, Erhard, Penzberg, Germany, Federal Republic of
Boehringer Mannheim GmbH, Mannheim, Germany, Federal Republic of (non-U.S. corporation)
US 6171842 B1 20010109
APPLICATION: US 2000-551028 20000417 (9)
PRIORITY: EP 1997-121232 19971203
DOCUMENT TYPE: Patent; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A process for recombinantly producing a protein, said protein comprising a first sequence and a second sequence C-terminal to the first sequence, the first sequence having the amino acid sequence of the first catalytic domain half of factor Xa and the second sequence having the amino acid sequence of the second catalytic domain half of trypsin, comprising expressing the protein in a host cell which contains an expression vector comprising a nucleic acid sequence which codes for the protein wherein the nucleic acid sequence is under the transcriptional control of a transcription control element, and isolating the expressed protein.

L7 ANSWER 8 OF 13 USPATFULL on STN
2000:167770 Chimeric serine proteases.
Bode, Wolfram, Gauting, Germany, Federal Republic of
Engh, Richard, Wessling, Germany, Federal Republic of
Hopfner, Karl-Peter, Munich, Germany, Federal Republic of
Huber, Robert, Germering, Germany, Federal Republic of
Kopetzki, Erhard, Penzberg, Germany, Federal Republic of
Boehringer Mannheim GmbH, Mannheim, Germany, Federal Republic of (non-U.S. corporation)
US 6159722 20001212
APPLICATION: US 1998-197801 19981123 (9)
PRIORITY: EP 1997-121232 19971203
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A chimeric protein comprising a first sequence and a second sequence C-terminal to the first sequence and linked to the first sequence by one or more peptide bonds, the first sequence having the amino acid sequence of the first catalytic domain half of a first serine protease and the second sequence having the amino acid sequence of the second catalytic domain half of a second serine protease different from the first serine protease; wherein the first sequence has the amino acid sequence of the first catalytic domain half of factor Xa and the second sequence has the amino acid sequence of the second catalytic domain half of trypsin.

2. The protein of claim 1 having the amino acid sequence corresponding to SEQ ID NO:12.

3. The protein of claim 1 in crystalline form.

L7 ANSWER 9 OF 13 USPATFULL on STN
2000:142131 Process for the production of peptides by way of streptavidin fusion proteins.
Kopetzki, Erhard, Penzberg, Germany, Federal Republic of
Roche Diagnostics GmbH, Mannheim, Germany, Federal Republic of (non-U.S. corporation)
US 6136564 20001024
WO 9718314 19970522
APPLICATION: US 1998-68738 19980625 (9)

WO 1996-EP4850 19961106 19980625 PCT 371 date 19980625 PCT 102(e) date
PRIORITY: DE 1995-19542702 19951116
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A process for recombinantly producing a peptide, the process comprising expressing, in a microorganism, a DNA which codes for a fusion protein comprising a streptavidin and the peptide to be produced, wherein the streptavidin and the peptide are linked via a cleavage sequence which is cleavable by a factor Xa or thrombin endoproteinase, to produce an insoluble fusion protein; isolating the insoluble fusion protein; solubilizing the fusion protein in a solution comprising a denaturing agent and adjusting the solution to a pH value of between 8.5 and 11; cleaving the fusion protein via the cleavage sequence using a factor X or thrombin endoproteinase to produce streptavidin cleavage segments and peptide cleavage segments; precipitating the streptavidin cleavage segments and uncleaved fusion protein from the solution by lowering the pH value of the solution; and purifying the peptide from the solution supernatant.

2. The process of claim 1, wherein the microorganism is prokaryotic.

3. The process of claim 2, wherein the insoluble fusion protein is isolated in inactive form.

4. The process of claim 3, wherein the insoluble fusion protein is isolated in an inclusion body.

5. The process of claim 1, wherein, in said precipitating step, the pH value of the solution is lowered below 6.

6. The process of claim 1, wherein the peptide to be produced is a natriuretic peptide or a parathyroid hormone peptide.

7. The process of claim 1, wherein the peptide to be produced is a urodilatin peptide or a parathyroid hormone peptide.

8. The process of claim 7, wherein the peptide to be produced is selected from the group consisting of a urodilatin peptide of amino acids 95-126 (SEO ID NO:2), a urodilatin peptide of amino acids 99-126 (amino acids 5-32 of SEQ ID NO:2), a urodilatin peptide of amino acids 102-126 (amino acids 8-32 of SEO ID NO:2) and a parathyroid hormone peptide of amino acids 1-37 (SEO ID NO:16).

9. The process of claim 1, wherein the cleavage sequence contains 5-15 amino acids and a C-terminal cleavage site.

10. The process of claim 9, wherein the cleavage sequence further contains a linker sequence comprising a plurality of amino acids attached at the N-terminal of the cleavage sequence, wherein each amino acid of the plurality of amino acids is independently selected from the group consisting of Gly, Thr, Ser, Ala, Pro, Asp, Glu, Arg and Lys.

11. The process of claim 10, wherein the linker sequence comprises 2-8 amino acids each of which is independently selected from the group consisting of Asp and Glu.

12. The process of claim 1, wherein the microorganism is eukaryotic.

13. The process of claim 1, wherein the solution is brought to a pH value of between 8,5 and 11 using art aqueous buffer.

L7 ANSWER 10 OF 13 USPATFULL on STN
97:89065 Recombinant core streptavidin.
Kopetzki, Erhard, Penzberg, Germany, Federal Republic of
Rudolph, Rainer, Weilheim, Germany, Federal Republic of
Grossmann, Adelbert, Eglfing, Germany, Federal Republic of
Boehringer Mannheim GmbH, Mannheim, Germany, Federal Republic of (non-U.S. corporation)
US 5672691 19970930
APPLICATION: US 1995-434718 19950504 (8)
PRIORITY: DE 1991-4135543 19911028
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

CLM What is claimed is:
1. A recombinant core streptavidin consisting of the amino acid sequence shown in SEQ ID NO: 2.

2. A method for immobilizing or detecting a target molecule in a diagnostic procedure or an affinity chromatography procedure comprising attaching a recombinant core streptavidin consisting of the amino acid sequence shown in SEQ ID NO: 2 to said target molecule and immobilizing or detecting said target molecule.

L7 ANSWER 11 OF 13 USPATFULL on STN
96:11074 Recombinant core-streptavidin.
Kopetzki, Erhard, Penzberg, Germany, Federal Republic of
Rudolph, Rainer, Weilheim, Germany, Federal Republic of
Grossmann, Adelbert, Eglfing, Germany, Federal Republic of
Boehringer Mannheim GmbH, Mannheim, Germany, Federal Republic of (non-U.S. corporation)
US 5489528 19960206
WO 9309144 19930513
APPLICATION: US 1994-211833 19940428 (8)
WO 1992-EP2463 19921028 19940428 PCT 371 date 19940428 PCT 102(e) date
PRIORITY: DE 1991-4135543 19911028
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A recombinant DNA which codes for a core streptavidin, consisting of (a) the nucleotide sequence shown in SEQ ID NO.1 or (b) a nucleotide sequence encoding the amino acid sequence encoded by SEQ ID NO:1.

2. A recombinant vector, comprising at least one copy of a DNA according to claim 1.

3. The vector according to claim 2, wherein said vector is a plasmid.

4. The vector according to claim 3, wherein said vector has a multicopy origin of replication.

5. The vector according to claim 2, wherein the DNA coding for the core streptavidin is under the control of a regulatable promoter.

6. A host cell transformed with a DNA according to claim 1.

7. A host cell transformed with a vector according to claim 2.

8. Plasmid pSAM-core DSM 6720.

L7 ANSWER 12 OF 13 USPATFULL on STN
95:64837 Process for the production and renaturation of recombinant, biologically active, eukaryotic alkaline phosphatase.
Michaelis, Uwe, Weilheim, Germany, Federal Republic of
Rudolph, Rainer, Weilheim, Germany, Federal Republic of
Jarsch, Michael, Bad Heilbrunn, Germany, Federal Republic of
Kopetzki, Erhard, Penzberg, Germany, Federal Republic of
Burtscher, Helmut, Habach, Germany, Federal Republic of
Schumacher, Gether, Bernried, Germany, Federal Republic of
Boehringer Mannheim GmbH, Mannheim-Waldhof, Germany, Federal Republic of (non-U.S. corporation)
US 5434067 19950718
APPLICATION: US 1993-100124 19930730 (8)
PRIORITY: DE 1992-42254272 19920731
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A process for the production of biologically active, eukaryotic alkaline phosphatase, comprising: a) expressing a DNA sequence coding for eukaryotic alkaline phosphatase in a prokaryotic host cell to produce eukaryotic alkaline phosphatase; b) lysing said prokaryotic host cell to obtain a lysate; c) isolating eukaryotic alkaline phosphatase from said lysate; d) solubilizing said isolated eukaryotic alkaline phosphatase under denaturing conditions in the presence of a reducing agent; and e) renaturing said isolated eukaryotic alkaline phosphatase in the presence of at least one stabilizing agent to obtain biologically active, eukaryotic alkaline phosphatase wherein said stabilizing agent

is present in an amount effective to stabilize said eukaryotic alkaline phosphatase and is selected from the group consisting of a sulfate salt, carbohydrate, or polyalcohol having at least 2 or more carbon atoms.

2. The process of claim 1 wherein said stabilizing agent is selected from the group consisting of at least one sulfate salt, carbohydrate, or polyalcohol having at least 2 carbon atoms.

3. The process of claim 1, wherein said stabilizing agent is at least one sulfate salt.

4. The process of claim 1 wherein said sulfate salt is at least one of the sulfate salts selected from the group consisting of sodium sulfate, potassium sulfate, or ammonium sulfate.

5. The process of claim 4 wherein said sulfate salt is sodium sulfate.

6. The process of claim 4 wherein said sulfate salt is potassium sulfate.

7. The process of claim 4, wherein sodium sulfate is added in an amount of 0.3-1 mol/l, potassium sulfate is added in an amount of 0.1-0.6 mol/l, and ammonium sulfate is added in an amount of 0.3-1 mol/l.

8. The process of claim 2, wherein said stabilizing agent is at least one carbohydrate.

9. The process of claim 8, wherein said carbohydrate is at least one of the carbohydrates selected from the group consisting of a pentose, hexose, or disaccharide.

10. The process of claim 8, wherein the total amount of carbohydrate added is 5 to 50% weight/volume relative to the volume of the renaturation mixture.

11. The process of claim 2, wherein said stabilizing agent is at least one polyalcohol, said polyalcohol having at least 2 carbon atoms.

12. The process of claim 11 wherein said polyalcohol is at least one of the polyalcohols selected from the group consisting of sorbitol, glycerol, erythritol, inositol, ethylene glycol.

13. The process of claim 11, wherein the total amount of polyalcohol added is 5 to 50% weight/volume relative to the volume of the renaturation mixture.

14. The process of claim 1, wherein the renaturation step is done in the presence of a mixture of a sulfate salt, a carbohydrate, and a polyalcohol having at least two carbon atoms.

15. The process of claim 1, wherein the renaturation step is done in the presence of a mixture of a sulfate salt and a polyalcohol having at least two carbon atoms.

16. The process of claim 1, wherein the renaturation step is done in the presence of a mixture of acarbohydrate and a polyalcohol having at least two carbon atoms.

17. The process of claim 1, wherein the renaturation step is done in the presence of a mixture of a sulfate salt and a carbohydrate.

18. The process of claim 17, wherein said sulfate salt is sodium sulfate and said carbohydrate is glycerol.

19. The process of claim 1, wherein the renaturation step is carried out in the presence of zinc ions and magnesium ions.

20. The process of claim 1, wherein the renaturation step is carried out in the presence of sulfhydryl reagents.

21. The process of claim 1, wherein the renaturation step is carried out in a pH range of 6 to 10.

22. The process of claim 1, wherein said prokaryotic host cell is gram negative.

23. The process of claim 22, wherein said prokaryotic host cell is E. coli.

24. The process of claim 1, wherein the DNA sequence coding for said eukaryotic alkaline phosphatase is introduced into said host cell by transformation with a vector which contains at least one copy of said DNA sequence.

25. The process of claim 24, wherein said vector is plasmid pPLAP (DSM 7094).

26. Biologically active, eukaryotic alkaline phosphatase obtained by the process of claim 1.

L7 ANSWER 13 OF 13 USPATFULL on STN
94:40195 Expression of HIV1 and HIV2 polypeptides and their use.
Bayer, Hubert, Weilheim, Germany, Federal Republic of
Kopetzki, Erhard, Penzberg, Germany, Federal Republic of
Boehringer Mannheim GmbH, Mannheim-Waldhof, United States (non-U.S. corporation)
US 5310876 19940510
APPLICATION: US 1991-648796 19910125 (7)
PRIORITY: DE 1990-4002636 19900130
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A fusion protein, comprising at least one antigenic or immunogenic determinant from a protein coded for by an HIV2 env region and having the amino acid sequence set forth in SEQ ID NO: 4, fused to a polypeptide not having an antigenic or immunogenic determinant, said polypeptide consisting essentially of an N-terminal sequence --NH_2 --Met--Tyr--Leu.

2. A fusion protein, comprising at least one antigenic or immunogenic determinant from a protein coded for by an HIV1 pol region and an HIV2 env region and having the amino acid sequence set forth in SEQ ID NO: 6, fused to a polypeptide not having an antigenic or immunogenic determinant, said polypeptide consisting essentially of an N-terminal sequence --NH_2 --Met--Tyr--Tyr--Leu.

3. A fusion protein, comprising at least one antigenic or immunogenic determinant from a protein coded for by an HIV2 env region, an HIV1 pol region, an HIV1 env region and an HIV1 gag region and having the amino acid sequence set forth in SEQ ID NO: 7, fused to a polypeptide not having an antigenic or immunogenic determinant, said polypeptide consisting essentially of an N-terminal sequence --NH_2 --Met--Tyr--Tyr--Leu.

4. A fusion protein, comprising at least one antigenic or immunogenic determinant from a protein coded for by an HIV2 env region, an HIV1 pol region, an HIV1 env region, an HIV1gag region, poly(Lys,Arg)n as a C-terminal amino acid sequence, and the amino acid sequence set forth in SEQ ID NO: 8, fused to a polypeptide not having an antigenic or immunogenic determinant, said polypeptide consisting essentially of an N-terminal sequence --NH_2 --Met--Tyr--Tyr--Leu.

=> d his

(FILE 'HOME' ENTERED AT 11:43:32 ON 08 JAN 2007)

FILE 'USPATFULL' ENTERED AT 11:43:44 ON 08 JAN 2007
E KACZMAREK ALEXANDRA/IN
L1 1 S E3
E KOPETZKI ERHARD/IN
L2 26 S E3
L3 16 S L2 AND (INCLUSION BODIES)
L4 3 S L3 AND (INCLUSION BODIES/CLM)
L5 2 S L4 NOT L1
L6 13 S L3 NOT L4
L7 13 S L6 AND AY<2003

```
=> e schantz christian/in
E1            2     SCHANTZ BRAD L/IN
E2            1     SCHANTZ CHRIS/IN
E3            2 --> SCHANTZ CHRISTIAN/IN
E4           27     SCHANTZ CHRISTOPHER A/IN
E5            1     SCHANTZ CURTIS/IN
E6            6     SCHANTZ DANIEL G/IN
E7            2     SCHANTZ DAVID L/IN
E8            1     SCHANTZ DECEASED SPENCER C/IN
E9            3     SCHANTZ ERIC J/IN
E10           7     SCHANTZ HANS G/IN
E11          20     SCHANTZ HANS GREGORY/IN
E12           2     SCHANTZ II ALLEN R/IN

=> s e3
L8            2 "SCHANTZ CHRISTIAN"/IN

=> s l8 not (l1 or l2)
L9            0 L8 NOT (L1 OR L2)

=> e seeber stefan/in
E1            1     SEEBER ROBERT R/IN
E2            2     SEEBER SIEGFRIED/IN
E3            6 --> SEEBER STEFAN/IN
E4            1     SEEBER TIMOTHY HOWARD/IN
E5            1     SEEBERG BJ O SLASHED RN ERIK/IN
E6            2     SEEBERG BJORN ERIK/IN
E7            1     SEEBERG CATO/IN
E8            1     SEEBERG ELVERFELD JENS/IN
E9            1     SEEBERG ELVERFELDT HERBERT/IN
E10           2     SEEBERG HORST/IN
E11           1     SEEBERG JOHN/IN
E12           2     SEEBERGER ACHIM/IN

=> s e3
L10           6 "SEEBER STEFAN"/IN

=> s l10 not (l1 or l2)
L11           5 L10 NOT (L1 OR L2)

=>  d l11,ti,1-5

L11  ANSWER 1 OF 5  USPATFULL on STN
TI     Antibodies against IL-13 receptor alpha1 and uses thereof

L11  ANSWER 2 OF 5  USPATFULL on STN
TI     Interferon-alpha polypeptides and conjugates

L11  ANSWER 3 OF 5  USPATFULL on STN
TI     DNA vectors without a selection marker gene

L11  ANSWER 4 OF 5  USPATFULL on STN
TI     Plasmids without a selection marker gene

L11  ANSWER 5 OF 5  USPATFULL on STN
TI     Recombinant restriction enzyme Sau3AI


=> file wpids
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                      ENTRY    SESSION
FULL ESTIMATED COST                                   45.23      45.44

FILE 'WPIDS' ENTERED AT 11:49:15 ON 08 JAN 2007
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FILE LAST UPDATED:                      2 JAN 2007   <20070102/UP>
MOST RECENT THOMSON SCIENTIFIC UPDATE:     200701     <200701/DW>
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    loaded into the database during the first half of January 2007.
    There will not be any update date (UP) written for the reclassified
```

documents, but they can be identified by 20060101/UPIC. <<<

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http://scientific.thomson.com/media/scpdf/ipcrdwpi.pdf

>>> FOR DETAILS ON THE NEW AND ENHANCED DERWENT WORLD PATENTS INDEX
PLEASE SEE
http://www.stn-international.de/stndatabases/details/dwpi_r.html <<<

```
=> e kaczmarek a/in
E1            1     KACZMARECK J S/IN
E2          200     KACZMAREK/IN
E3           15 --> KACZMAREK A/IN
E4            1     KACZMAREK A A/IN
E5            2     KACZMAREK A D/IN
E6            5     KACZMAREK A R/IN
E7            5     KACZMAREK B/IN
E8            1     KACZMAREK B D/IN
E9            6     KACZMAREK D/IN
E10           4     KACZMAREK E/IN
E11           8     KACZMAREK F/IN
E12           5     KACZMAREK G/IN

=> s e3
L12          15 "KACZMAREK A"/IN

=> s l12 and (inclusion bodies)
         25159 INCLUSION
         83261 BODIES
           546 INCLUSION BODIES
                 (INCLUSION(W)BODIES)
L13            1 L12 AND (INCLUSION BODIES)

=> d l13,bib,ab

L13  ANSWER 1 OF 1  WPIDS COPYRIGHT 2007       THE THOMSON CORP on STN
Full Text
AN   2004-379041 [36]   WPIDS
DNC  C2004-142282 [36]
TI   Recombinantly producing antifusogenic peptides, useful for inhibiting
     viral fusion to cell membranes, comprises expressing a nucleic acid
     encoding the antifusogenic peptide as a repeat peptide in microbial host
     cells
DC   B04; D16
IN   KACZMAREK A; KOPETZKI E; SCHANTZ C; SEEBER S; ALEXANDRA K; CHRISTIAN S;
     ERHARD K
PA   (HOFF-C) HOFFMANN LA ROCHE & CO AG F; (KACZ-I) KACZMAREK A; (KOPE-I)
     KOPETZKI E; (SCHA-I) SCHANTZ C; (SEEB-I) SEEBER S
CYC  38
PIA  EP 1422237      A2 20040526 (200436)* EN  22[1]
     CA 2443365      A1 20040519 (200438)  EN
     JP 2004166703   A  20040617 (200440)  JA  22
     CN 1502698      A  20040609 (200460)  ZH
     KR 2004044372   A  20040528 (200463)  KO
     MX 2003010433   A1 20041001 (200557)  ES
     US 20050191729  A1 20050901 (200558)  EN
     IN 2003000944   I4 20051230 (200604)  EN
     CN 1254542      C  20060503 (200661)  ZH
ADT  EP 1422237 A2 EP 2003-26521 20031118; CA 2443365 A1 CA 2003-2443365
     20031008; MX 2003010433 A1 MX 2003-10433 20031114; IN 2003000944 I4 IN
     2003-CH944 20031117; US 20050191729 A1 US 2003-716095 20031118; CN 1502698
     A CN 2003-10116190 20031119; JP 2004166703 A JP 2003-389592 20031119; KR
     2004044372 A KR 2003-82062 20031119; CN 1254542 C CN 2003-10116190
     20031119
```

PRAI EP 2003-988 20030117
EP 2002-25618 20021119
AB EP 1422237 A2 UPAB: 20060121
NOVELTY - A process for the recombinant production of an antifusogenic peptide comprises expressing a nucleic acid encoding the antifusogenic peptide as a repeat peptide in a microbial host cell and isolating the **inclusion bodies** containing the repeat peptide.

DETAILED DESCRIPTION - A process for the recombinant production of an antifusogenic peptide comprising:

(a) expressing a nucleic acid encoding the antifusogenic peptide as repeat peptide in a microbial host cell;

(b) isolating the **inclusion bodies** containing the repeat peptide;

(c) solubilizing the **inclusion bodies**; and

(d) isolating the antifusogenic peptide after cleavage,

characterized by washing the **inclusion bodies** with a denaturing agent at a pH value of or below pH 6.5, solubilizing the **inclusion bodies** containing the repeat peptide at a pH value of at least pH 9 and cleaving the repeat peptide to obtain the antifusogenic peptide.

INDEPENDENT CLAIMS are also included for the following:

(1) a nucleic acid encoding a fusion polypeptide consisting of (in N-terminal to C-terminal direction): (a) an antifusogenic peptide as repeat peptide of at least 2 identical antifusogenic peptide sequences; and (b) a cleavage site located between the antifusogenic peptides; and

(2) a preparation of **inclusion bodies** containing a fusion polypeptide consisting of (in N-terminal to C-terminal direction): (a) an antifusogenic peptide as repeat peptide of a length of about 10-100 amino acids; and (b) a cleavage site located between the antifusogenic peptides.

ACTIVITY - Virucide.

MECHANISM OF ACTION - Peptidic Inhibitor.

USE - The method is useful for producing antifusogenic peptides (claimed) which inhibit the fusion of viruses with membranes of target cells, particularly peptidic inhibitors of HIV, simian immunodeficiency virus, measles virus, and influenza viruses.

ADVANTAGE - Unlike previous method of producing peptides, the new method enables the recombinant production of high yield of antifusogenic peptides via the inclusion body route, and is suitable for the large-scale industrial production of such peptides.

=> d his

(FILE 'HOME' ENTERED AT 11:43:32 ON 08 JAN 2007)

FILE 'USPATFULL' ENTERED AT 11:43:44 ON 08 JAN 2007

```
                E KACZMAREK ALEXANDRA/IN
L1            1 S E3
                E KOPETZKI ERHARD/IN
L2           26 S E3
L3           16 S L2 AND (INCLUSION BODIES)
L4            3 S L3 AND (INCLUSION BODIES/CLM)
L5            2 S L4 NOT L1
L6           13 S L3 NOT L4
L7           13 S L6 AND AY<2003
                E SCHANTZ CHRISTIAN/IN
L8            2 S E3
L9            0 S L8 NOT (L1 OR L2)
                E SEEBER STEFAN/IN
L10           6 S E3
L11           5 S L10 NOT (L1 OR L2)
```

FILE 'WPIDS' ENTERED AT 11:49:15 ON 08 JAN 2007

```
                E KACZMAREK A/IN
L12          15 S E3
L13           1 S L12 AND (INCLUSION BODIES)
```

=> e kopetzki e/in

```
E1            1     KOPETZK R/IN
E2           33     KOPETZKI/IN
E3           30 --> KOPETZKI E/IN
E4            2     KOPETZKI M/IN
E5            1     KOPETZKI N/IN
E6          106     KOPETZKY/IN
E7            1     KOPETZKY C A/IN
E8            2     KOPETZKY C D/IN
E9            1     KOPETZKY C R/IN
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E10          1     KOPETZKY E/IN
E11         21     KOPETZKY H/IN
E12          1     KOPETZKY M/IN

=> s e3
L14         30 "KOPETZKI E"/IN

=> s l14 and (inclusion bodies)
         25159 INCLUSION
         83261 BODIES
           546 INCLUSION BODIES
                 (INCLUSION(W)BODIES)
L15          9 L14 AND (INCLUSION BODIES)

=> s l15 not l12
L16          8 L15 NOT L12

=> d l16,ti,1-8

L16  ANSWER 1 OF 8  WPIDS COPYRIGHT 2007       THE THOMSON CORP on STN

TI   Recombinantly producing a desired polypeptide for increasing the yield of
     insoluble polypeptides, comprises expressing a nucleic acid encoding the
     polypeptide in a microbial host cell, and forming inclusion bodies

L16  ANSWER 2 OF 8  WPIDS COPYRIGHT 2007       THE THOMSON CORP on STN

TI   Autocatalytically cleavable zymogenic protease precursors - useful for
     cleaving fusion proteins and for therapeutic uses

L16  ANSWER 3 OF 8  WPIDS COPYRIGHT 2007       THE THOMSON CORP on STN

TI   Autocatalytically cleavable, inactive protease precursors - used for
     production of therapeutic proteins from their fusion proteins

L16  ANSWER 4 OF 8  WPIDS COPYRIGHT 2007       THE THOMSON CORP on STN

TI   Non-glycosylated, truncated forms of factor IX family protein with serine
     protease activity - used to screen for specific modulators and to assay
     factor IXa

L16  ANSWER 5 OF 8  WPIDS COPYRIGHT 2007       THE THOMSON CORP on STN

TI   Recombinant production of peptide(s) as fusions with streptavidin attached
     via cleavable linker - especially for urotropin and parathyroid hormone
     production

L16  ANSWER 6 OF 8  WPIDS COPYRIGHT 2007       THE THOMSON CORP on STN

TI   Recombinant production of natriuretic peptide as streptavidin fusion
     protein - useful for preparation of urodilatin which is used to treat
     acute kidney disease

L16  ANSWER 7 OF 8  WPIDS COPYRIGHT 2007       THE THOMSON CORP on STN

TI   Isolation of recombinant eukaryotic alkaline phosphatase - from
     prokaryotic cells by lysis, solubilisation and renaturation in presence of
     stabiliser

L16  ANSWER 8 OF 8  WPIDS COPYRIGHT 2007       THE THOMSON CORP on STN

TI   Expressing recombinant protein in soluble form - under control of
     inducible promoter by limiting induction, to reduce transcription rate and
     prevent formation of inclusion bodies


=> d l16,bib,ab,1

L16  ANSWER 1 OF 8  WPIDS COPYRIGHT 2007       THE THOMSON CORP on STN
Full Text
AN   2005-015092 [02]   WPIDS
DNC  C2005-004640 [02]
TI   Recombinantly producing a desired polypeptide for increasing the yield of
     insoluble polypeptides, comprises expressing a nucleic acid encoding the
     polypeptide in a microbial host cell, and forming inclusion bodies
```

DC B04; D16
IN GRUENBECK R; **KOPETZKI E**; POPP F; GURENBECK R
PA (GRUE-I) GRUENBECK R; (HOFF-C) HOFFMANN LA ROCHE & CO AG F; (KOPE-I) KOPETZKI E; (POPP-I) POPP F; (HOFF-C) HOFFMANN LA ROCHE INC
CYC 37
PIA EP 1486571 A1 20041215 (200502)* EN 11[0]
    JP 2005000170 A 20050106 (200504) JA 9
    US 20050003485 A1 20050106 (200504) EN
    CA 2467142 A1 20041212 (200505) EN
    KR 2004107397 A 20041220 (200526) KO
    CN 1572872 A 20050202 (200532) ZH
    EP 1486571 B1 20051214 (200602) EN
    DE 60302776 E 20060119 (200614) DE
    IN 2004000530 I4 20060203 (200619) EN
    US 7034119 B2 20060425 (200628) EN
    ES 2253604 T3 20060601 (200638) ES
    JP 3783963 B2 20060607 (200638) JA 9
    DE 60302776 T2 20060817 (200655) DE
ADT EP 1486571 A1 EP 2003-12295 20030612; DE 60302776 E DE 2003-602776 20030612; DE 60302776 E EP 2003-12295 20030612; ES 2253604 T3 EP 2003-12295 20030612; CA 2467142 A1 CA 2004-2467142 20040512; IN 2004000530 I4 IN 2004-CH530 20040608; JP 2005000170 A JP 2004-171458 20040609; JP 3783963 B2 JP 2004-171458 20040609; CN 1572872 A CN 2004-10049377 20040610; KR 2004107397 A KR 2004-42867 20040611; US 20050003485 A1 US 2004-866567 20040612; US 7034119 B2 US 2004-866567 20040612; DE 60302776 T2 DE 2003-602776 20030612; DE 60302776 T2 EP 2003-12295 20030612
FDT DE 60302776 E Based on EP 1486571 A; ES 2253604 T3 Based on EP 1486571 A; JP 3783963 B2 Previous Publ JP 2005000170 A; DE 60302776 T2 Based on EP 1486571 A
PRAI EP 2003-12295 20030612
AB EP 1486571 A1 UPAB: 20060203
NOVELTY - Recombinantly producing a desired polypeptide comprises expressing a nucleic acid encoding the polypeptide in a microbial host cell, forming **inclusion bodies** containing the polypeptide, and isolating, solubilizing and naturing the polypeptide, where after fermentation the host cell or the host cell content is incubated at a temperature of 40 degrees C or higher for 10 minutes.

DETAILED DESCRIPTION - Recombinantly producing a desired polypeptide comprises expressing a nucleic acid encoding the polypeptide in a microbial host cell, forming **inclusion bodies** containing the polypeptide, and isolating, solubilizing and naturing the polypeptide, where after fermentation the host cell or the host cell content is incubated at a temperature of 40 degrees C or higher for 10 minutes and subsequently the insoluble polypeptide is isolated from the host cell.

USE - The methods and compositions of the present invention are useful for increasing the yield of insoluble polypeptides in the form of **inclusion bodies** after recombinant expression

```
=> e schantz c/in
E1          171     SCHANTZ/IN
E2            1     SCHANTZ A R/IN
E3            5 --> SCHANTZ C/IN
E4           28     SCHANTZ C A/IN
E5            1     SCHANTZ C S/IN
E6            6     SCHANTZ D G/IN
E7           10     SCHANTZ D L/IN
E8            4     SCHANTZ E J/IN
E9            4     SCHANTZ H/IN
E10          23     SCHANTZ H G/IN
E11           3     SCHANTZ J/IN
E12           4     SCHANTZ J L/IN

=> s e3
L17           5 "SCHANTZ C"/IN

=> d l17,ti,1-5

L17  ANSWER 1 OF 5  WPIDS COPYRIGHT 2007        THE THOMSON CORP on STN

TI   New nucleic acid (having codons cytosine-guanine-thymine at positions 33, 35 and 36) encoding alpha-chain of hepatocyte growth factor or N-terminal fragment, useful to produce alpha-chain of hepatocyte growth factor or N-terminal fragment
```

```
L17  ANSWER 2 OF 5  WPIDS COPYRIGHT 2007       THE THOMSON CORP on STN

TI   Recombinantly producing antifusogenic peptides, useful for inhibiting
     viral fusion to cell membranes, comprises expressing a nucleic acid
     encoding the antifusogenic peptide as a repeat peptide in microbial host
     cells

L17  ANSWER 3 OF 5  WPIDS COPYRIGHT 2007       THE THOMSON CORP on STN

TI   Electronic information publishing method for Internet, involves
     transforming information into MVR directory and storing it in document
     server so as to be transmitted to recipient

L17  ANSWER 4 OF 5  WPIDS COPYRIGHT 2007       THE THOMSON CORP on STN

TI   Novel prokaryotic expression system, used to produce recombinant proteins

L17  ANSWER 5 OF 5  WPIDS COPYRIGHT 2007       THE THOMSON CORP on STN

TI   Thermal ink jet printer head substrate  - has plastic base, metal layer
     and dielectric layer


=> e seeber s/in
E1            1     SEEBER P K/IN
E2            6     SEEBER R/IN
E3           19 --> SEEBER S/IN
E4            1     SEEBER T H/IN
E5            2     SEEBER V/IN
E6            4     SEEBER W/IN
E7           19     SEEBERG/IN
E8            2     SEEBERG A/IN
E9            5     SEEBERG B E/IN
E10           2     SEEBERG B R E/IN
E11           1     SEEBERG E/IN
E12           3     SEEBERG H/IN

=> s e3
L18          19 "SEEBER S"/IN

=> d his

     (FILE 'HOME' ENTERED AT 11:43:32 ON 08 JAN 2007)

     FILE 'USPATFULL' ENTERED AT 11:43:44 ON 08 JAN 2007
                  E KACZMAREK ALEXANDRA/IN
L1              1 S E3
                  E KOPETZKI ERHARD/IN
L2             26 S E3
L3             16 S L2 AND (INCLUSION BODIES)
L4              3 S L3 AND (INCLUSION BODIES/CLM)
L5              2 S L4 NOT L1
L6             13 S L3 NOT L4
L7             13 S L6 AND AY<2003
                  E SCHANTZ CHRISTIAN/IN
L8              2 S E3
L9              0 S L8 NOT (L1 OR L2)
                  E SEEBER STEFAN/IN
L10             6 S E3
L11             5 S L10 NOT (L1 OR L2)

     FILE 'WPIDS' ENTERED AT 11:49:15 ON 08 JAN 2007
                  E KACZMAREK A/IN
L12            15 S E3
L13             1 S L12 AND (INCLUSION BODIES)
                  E KOPETZKI E/IN
L14            30 S E3
L15             9 S L14 AND (INCLUSION BODIES)
L16             8 S L15 NOT L12
                  E SCHANTZ C/IN
L17             5 S E3
                  E SEEBER S/IN
L18            19 S E3

=> s l18 not (l12 or l14)
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L19         18 L18 NOT (L12 OR L14)

=> s l19 and (inclusion bodies)
         25159 INCLUSION
         83261 BODIES
           546 INCLUSION BODIES
                 (INCLUSION(W)BODIES)
L20          2 L19 AND (INCLUSION BODIES)

=> d l20,bib,ab,1-2

L20  ANSWER 1 OF 2  WPIDS COPYRIGHT 2007       THE THOMSON CORP on STN
Full Text
AN   2006-027231 [03]   WPIDS
DNC  C2006-009261 [03]
DNN  N2006-023720 [03]
TI   Novel isolated or recombinant interferon-alpha polypeptide exhibiting
     antiviral activity, useful for reducing number of copies of virus in cells
     infected with virus
DC   A25; A96; B04; D16; Q57
IN   ADAM S S; DEMBOWSKI M; FALKENSTEIN R; FISCHER S; GROSSMANN A; HESSE F;
     KOLL H; KROPSHOFER H; NISSEN T L; PATTEN P A; SCHAUBMAR A; SCHUMACHER R;
     SEEBER S; VISWANATHAN S; VOGT A
PA   (HOFF-C) HOFFMANN LA ROCHE & CO AG F; (MAXY-N) MAXYGEN INC; (ROCH-N) ROCHE
     PALO ALTO LLC
CYC  109
PIA  US 20050266465  A1 20051201 (200603)* EN  123[9]
     WO 2005113592   A2 20051201 (200603)  EN
ADT  US 20050266465 A1 Provisional US 2004-572504P 20040519; US 20050266465 A1
     US 2005-132722 20050518; WO 2005113592 A2 WO 2005-US17471 20050518
PRAI US 2005-132722 20050518
     US 2004-572504P 20040519
AB   US 20050266465 A1   UPAB: 20060112
      NOVELTY - An isolated or recombinant interferon (IFN)-alpha polypeptide
     (I) comprising a sequence differing in 1-16 amino acid positions from a
     fully defined 166 amino acid (SEQ ID No. 1) sequence given in the
     specification, and comprising one or more substitution relative to SEQ ID
     No. 1 chosen from F48A/L, V51P, F55A, F65A, F68P, L111A and V114P, where
     the polypeptide exhibiting antiviral activity, is new.
          DETAILED DESCRIPTION - An isolated or recombinant interferon
     (IFN)-alpha polypeptide (I), is chosen from:
          (a) a polypeptide comprising a sequence differing in 1-16 amino
     acid positions from a fully defined 166 amino acid (SEQ ID No. 1) sequence
     given in the specification, and comprising one or more substitution
     relative to SEQ ID No. 1 chosen from F48A/L, V51P, F55A, F65A, F68P, L111A
     and V114P, where the polypeptide exhibits antiviral activity;
          (b) a polypeptide comprising a sequence differing in 0-16 amino
     acid positions from a fully defined 166 amino acid (SEQ ID No. 13)
     sequence given in the specification, and comprising one or more of Ala or
     Leu at position 48, Pro at position 51, Ala at position 55, Ala at
     position 65, Pro at position 68, Ala at position 111, and Pro at position
     114, where the polypeptide exhibits antiviral activity;
          (c) a polypeptide comprising the sequence of SEQ ID No. 13,
     optionally further comprising a methionine at the N-terminus, where the
     polypeptide exhibits antiviral activity;
          (d) a polypeptide comprising a sequence differing in 1-16 amino
     acid positions from a fully defined 166 amino acid (SEQ ID No. 36)
     sequence given in the specification, and comprising one or more
     substitution relative to SEQ ID No. 36 chosen from M21A, I24P, F48A/L,
     T51P, S55A, F65A, F68P, F90A, M93P, L111A, V114P, F124A, I127P and E160D,
     where the polypeptide exhibits antiviral antibody;
          (e) a polypeptide comprising a sequence differing in 0-16 amino
     acid positions from a fully defined 166 amino acid (SEQ ID No. 38)
     sequence given in the specification, and comprising one or more of Ala at
     position 21, Pro at position 24, Ala or Leu at position 48, Pro at
     position 51, Ala at position 55, Ala at position 65, Pro at position 68,
     Ala at position 90, Pro at position 93, Ala at position 111, Pro at
     position 114, Ala at position 124, Pro at position 127 and Glu at position
     160, where the polypeptide exhibits antiviral activity; and
          (f) a polypeptide comprising the sequence of SEQ ID No. 38,
     optionally further comprising a methionine at the N-terminus, where the
     polypeptide exhibits antiviral activity.
          INDEPENDENT CLAIMS are also included for:
          (1) a conjugate (II) chosen from:
          (a) a conjugate comprising the polypeptide as mentioned in (b) or
```

(e) of (I), and a non-polypeptide moiety covalently attached to (I), where the conjugate exhibits antiviral activity;

(b) a conjugate comprising the polypeptide as mentioned in (c) of (I), and a polyethylene glycol (PEG) moiety covalently attached to a lysine residue of (I), where the conjugate exhibits antiviral activity, and

(c) a conjugate comprising the polypeptide as mentioned in (f) of (I), and a PEG moiety covalently attached to a lysine residue of (I), where the conjugate exhibits antiviral activity;

(2) a composition (C1) chosen from:

(a) a composition comprising a conjugate having the polypeptide as mentioned in (c) of (I), and a 40 kDa monomethoxy PEG2 (mPEG2) moiety covalently attached to Lys122, and a conjugate comprising a polypeptide as mentioned in (c) of (I) and a 40 kDa mPEG2 moiety covalently attached to Lys135, where the composition exhibits antiviral activity;

(b) a composition comprising a conjugate having a polypeptide as mentioned in (f) of (I), and a 40 kDa mPEG2 moiety covalently attached to Lys31, a conjugate comprising a polypeptide as mentioned in (f) of (I), and a 40 kDa mPEG2 moiety covalently attached to Lys122, and a conjugate comprising a polypeptide as mentioned in (f) of (I), and a 40 kDa mPEG2 moiety covalently attached to Lys135, where the composition exhibits antiviral activity;

(c) a composition comprising the polypeptide as mentioned in (b) or (e) of (I), and an excipient, and

(d) a composition comprising the conjugate as mentioned in (b) of (II), and an excipient;

(3) an isolated or recombinant polynucleotide (III) comprising a nucleic acid sequence encoding the polypeptide as mentioned in (b) or (e) of (I);

(4) a vector (IV) comprising (III);

(5) a host cell (V) comprising (III) or (IV);

(6) a composition comprising (III) and an excipient;

(7) preparing (I), being the polypeptide as mentioned in (b) or (e) of (I); and

(8) preparing (M1) (II), involves providing the polypeptide as mentioned in (b) or (e) of (I), and attaching at least one non-polypeptide moiety to (I), where the resulting conjugate exhibits antiviral activity.

ACTIVITY - Virucide; Antiinflammatory; Hepatotropic; Anti-HIV; Cytostatic.

In vitro analysis of the efficacy of interferon (IFN)-alpha polypeptide in exhibiting antiproliferative activity was carried out as follows. Human Burkitt's lymphoma cells (Daudi cells) were transferred to T175 tissue culture flasks containing complete RPMI (50 ml) and maintained at 37 degreesC in a humidified 5% carbon dioxide incubator. The cells were spun down and washed with 1X phosphate buffered saline (PBS). The cell number was adjusted to 105 cells/ml. The culture medium (80 mul) was added to each well in 96-well round bottom assay plates followed by transfer of cells (100 mul). The IFN-alpha (20 mul) was added to the assay plate. The cells were incubated at 37degreesC in a humidified 5% carbon dioxide incubator. The viability of the cells was assayed. The result indicates antiproliferative activity of the IFN-alpha polypeptide.

MECHANISM OF ACTION - Gene therapy.

USE - (I) is useful for reducing the number of copies of a virus in cells infected with the virus, and reducing the level of hepatitis C virus (HCV) RNA, HBV DNA or HIV RNA in the serum of a patient infected with HCV, HBV or HIV.

(II) is useful for reducing the number of copies of a virus in cells infected with the virus, which involves administering (II) to the cells to reduce the number of copies of the virus in the cells, thus reducing the number of copies of the virus in the cells. (II) is useful for reducing the level of HCV RNA, HBV DNA or HIV RNA in the serum of a patient infected with HCV, HBV or HIV, which involves administering (II) to the patient to reduce the level of HCV RNA, HBV DNA or HIV RNA, compared to the HCV RNA level, HBV DNA level or HIV RNA level present prior to the start of treatment (claimed).

(I) is useful for treating hairy cell leukemia, malignant melanoma, follicular lymphoma, non-Hodgkin's lymphoma, bladder cancer, Crohn's disease, AIDS, etc. (I) is useful for enhancing TH1 differentiation of TH0 cells.

ADVANTAGE - (I) has enhanced antiviral and/or immunomodulatory efficacy.

L20 ANSWER 2 OF 2 WPIDS COPYRIGHT 2007 THE THOMSON CORP on STN
Full Text
AN 2005-690246 [71] WPIDS

DNC C2005-209921 [71]
TI New nucleic acid (having codons cytosine-guanine-thymine at positions 33, 35 and 36) encoding alpha-chain of hepatocyte growth factor or N-terminal fragment, useful to produce alpha-chain of hepatocyte growth factor or N-terminal fragment
DC B04; D16
IN AUER J; PAPADIMITRIOU A; SCHANTZ C; **SEEBER S**
PA (HOFF-C) HOFFMANN LA ROCHE & CO AG F
CYC 108
PIA WO 2005095611 A1 20051013 (200571)* EN 27[1]
EP 1723236 A1 20061122 (200677) EN
ADT WO 2005095611 A1 WO 2005-EP2176 20050302; EP 1723236 A1 EP 2005-715653 20050302; EP 1723236 A1 WO 2005-EP2176 20050302
FDT EP 1723236 A1 Based on WO 2005095611 A
PRAI EP 2004-4951 20040303
AB WO 2005095611 A1 UPAB: 20051223
NOVELTY - Nucleic acid (I) encoding the alpha-chain of hepatocyte growth factor or an N-terminal fragment, where at least one of the codons of amino acids (codons at positions 33, 35 and 36) is cytosine-guanine-thymine (CGT), is new.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a method for the production of alpha-chain of hepatocyte growth factor or an N-terminal fragment (NK polypeptide) by expression of a nucleic acid encoding the NK polypeptide in a microbial host cell, isolating of **inclusion bodies** containing the NK polypeptide in denatured form, solubilization of the **inclusion bodies** and naturation of the denatured NK polypeptide, where in the nucleic acid at least one of the codons of amino acids is codons at positions 33, 35 and 36 is CGT.

USE - (I) is useful for the production alpha-chain of hepatocyte growth factor or an N-terminal fragment (claimed).

ADVANTAGE - The modification of at least one of the codons of the DNA sequence of positions results in an improved expression yield of 100% or more. The amino acid 32 is changed from glutamine to serine to improve homogeneity of the protein (cleavage of N-terminal methionine).

=> d his

(FILE 'HOME' ENTERED AT 11:43:32 ON 08 JAN 2007)

FILE 'USPATFULL' ENTERED AT 11:43:44 ON 08 JAN 2007
E KACZMAREK ALEXANDRA/IN
L1 1 S E3
E KOPETZKI ERHARD/IN
L2 26 S E3
L3 16 S L2 AND (INCLUSION BODIES)
L4 3 S L3 AND (INCLUSION BODIES/CLM)
L5 2 S L4 NOT L1
L6 13 S L3 NOT L4
L7 13 S L6 AND AY<2003
E SCHANTZ CHRISTIAN/IN
L8 2 S E3
L9 0 S L8 NOT (L1 OR L2)
E SEEBER STEFAN/IN
L10 6 S E3
L11 5 S L10 NOT (L1 OR L2)

FILE 'WPIDS' ENTERED AT 11:49:15 ON 08 JAN 2007
E KACZMAREK A/IN
L12 15 S E3
L13 1 S L12 AND (INCLUSION BODIES)
E KOPETZKI E/IN
L14 30 S E3
L15 9 S L14 AND (INCLUSION BODIES)
L16 8 S L15 NOT L12
E SCHANTZ C/IN
L17 5 S E3
E SEEBER S/IN
L18 19 S E3
L19 18 S L18 NOT (L12 OR L14)
L20 2 S L19 AND (INCLUSION BODIES)

=> file medline
COST IN U.S. DOLLARS SINCE FILE ENTRY TOTAL SESSION

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FULL ESTIMATED COST                                  48.88      94.32

FILE 'MEDLINE' ENTERED AT 11:53:20 ON 08 JAN 2007

 FILE LAST UPDATED: 6 Jan 2007 (20070106/UP).  FILE COVERS 1950 TO DATE.

  All regular MEDLINE updates from November 15 to December 16 have been
  added to MEDLINE, along with 2007 Medical Subject Headings (MeSH(R))
  and 2007 tree numbers.

  The annual reload will be available in early 2007.

  This file contains CAS Registry Numbers for easy and accurate
  substance identification.

=> e (kaczmarek a/au)
'AU)' IS NOT A VALID EXPAND FIELD CODE FOR FILE 'MEDLINE'
The indicated field code is not available for EXPAND in this
file.  To see a list of valid EXPAND field codes, enter HELP
SFIELDS at an arrow prompt (=>).

=> e kaczmarek a/au
E1            1     KACZMARECK A/AU
E2            3     KACZMARECK H/AU
E3           49 --> KACZMAREK A/AU
E4            2     KACZMAREK A T/AU
E5           11     KACZMAREK AGNIESZKA/AU
E6            1     KACZMAREK ALDONA/AU
E7            2     KACZMAREK ANDRZEJ/AU
E8            4     KACZMAREK ANNA/AU
E9           25     KACZMAREK B/AU
E10           3     KACZMAREK B L/AU
E11           1     KACZMAREK BARBARA/AU
E12           3     KACZMAREK BARTOSZ/AU

=> s e3
L21          49 "KACZMAREK A"/AU

=> s l21 and (inclusion bodies)
         66032 INCLUSION
        109790 BODIES
         17324 INCLUSION BODIES
                 (INCLUSION(W)BODIES)
L22           0 L21 AND (INCLUSION BODIES)

=> d l21,ti,1-5

L21  ANSWER 1 OF 49     MEDLINE on STN
TI   Anaemia is an independent predictor of poor outcome in patients with
     chronic heart failure.

L21  ANSWER 2 OF 49     MEDLINE on STN
TI   Humoral responses to melanoma vaccine, genetically modified with
     interleukin 6 and soluble interleukin 6 receptor.

L21  ANSWER 3 OF 49     MEDLINE on STN
TI   Antibiotic sensitivity of bacteria isolated in 1998 and 1999 from patients
     with infections of upper airways residing in Western Pomerania.

L21  ANSWER 4 OF 49     MEDLINE on STN
TI   [Investigations on the prevalence of headache associated with mandibular
     dysfunction].
     Badania nad czestoscia wystepowania bolow glowy zwiazanych z dysfunkcja
     ukladu ruchowego narzadu zucia.

L21  ANSWER 5 OF 49     MEDLINE on STN
TI   Genetically modified tumour vaccines (GMTV) in melanoma clinical trials.


=> e kopetzki e/au
E1            2     KOPETZ V/AU
E2            1     KOPETZKI C/AU
E3           15 --> KOPETZKI E/AU
E4            4     KOPETZKI ERHARD/AU
E5            1     KOPETZKI KOGLER U/AU
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E6            4      KOPETZKY C D/AU
E7            1      KOPETZKY GERHARD/AU
E8           15      KOPETZKY M T/AU
E9            1      KOPETZKY S/AU
E10           2      KOPETZKY S J/AU
E11           1      KOPEVAN C/AU
E12           1      KOPEYAN/AU

=> s e3-e4
            15 "KOPETZKI E"/AU
             4 "KOPETZKI ERHARD"/AU
L23         19 ("KOPETZKI E"/AU OR "KOPETZKI ERHARD"/AU)

=> s l23 and (inclusion bodies)
         66032 INCLUSION
        109790 BODIES
         17324 INCLUSION BODIES
                (INCLUSION(W)BODIES)
L24          0 L23 AND (INCLUSION BODIES)

=> d l23,ti,1-5

L23  ANSWER 1 OF 19      MEDLINE on STN
TI   Physiological fIXa activation involves a cooperative conformational
     rearrangement of the 99-loop.

L23  ANSWER 2 OF 19      MEDLINE on STN
TI   The influence of residue 190 in the S1 site of trypsin-like serine
     proteases on substrate selectivity is universally conserved.

L23  ANSWER 3 OF 19      MEDLINE on STN
TI   Crystal structures of uninhibited factor VIIa link its cofactor and
     substrate-assisted activation to specific interactions.

L23  ANSWER 4 OF 19      MEDLINE on STN
TI   Structural basis of the adaptive molecular recognition by MMP9.

L23  ANSWER 5 OF 19      MEDLINE on STN
TI   Coagulation factor IXa: the relaxed conformation of Tyr99 blocks substrate
     binding.


=> e schantz c/au
E1           39      SCHANTZ B/AU
E2            1      SCHANTZ B J/AU
E3            2 --> SCHANTZ C/AU
E4            4      SCHANTZ C W/AU
E5            4      SCHANTZ D/AU
E6            1      SCHANTZ DUNN J/AU
E7            3      SCHANTZ E/AU
E8           40      SCHANTZ E J/AU
E9            5      SCHANTZ E T/AU
E10           1      SCHANTZ G/AU
E11           1      SCHANTZ G H/AU
E12           1      SCHANTZ H/AU

=> s e3
L25           2 "SCHANTZ C"/AU

=> d l25,ti,1-2

L25  ANSWER 1 OF 2      MEDLINE on STN
TI   Molecular pathogenesis of a disease: structural consequences of
     aspartylglucosaminuria mutations.

L25  ANSWER 2 OF 2      MEDLINE on STN
TI   The trouble with clinical trials.


=> e seeber s/au
E1            1      SEEBER RUTH/AU
E2            4      SEEBER RUTH M/AU
E3          329 --> SEEBER S/AU
E4            2      SEEBER S J/AU
E5           32      SEEBER SIEGFRIED/AU
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E6            1     SEEBER SIGFRIED/AU
E7            5     SEEBER SILKE/AU
E8            4     SEEBER U/AU
E9            7     SEEBERG A/AU
E10           6     SEEBERG A H/AU
E11           1     SEEBERG A M/AU
E12           1     SEEBERG ASTRID H/AU

=> s e3
L26         329 "SEEBER S"/AU

=> s l26 and (inclusion bodies)
          66032 INCLUSION
         109790 BODIES
          17324 INCLUSION BODIES
                  (INCLUSION(W)BODIES)
L27           0 L26 AND (INCLUSION BODIES)

=> file uspatful
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     1.17      95.49

FILE 'USPATFULL' ENTERED AT 11:55:20 ON 08 JAN 2007
CA INDEXING COPYRIGHT (C) 2007 AMERICAN CHEMICAL SOCIETY (ACS)

FILE COVERS 1971 TO PATENT PUBLICATION DATE: 4 Jan 2007 (20070104/PD)
FILE LAST UPDATED: 4 Jan 2007 (20070104/ED)
HIGHEST GRANTED PATENT NUMBER: US7159245
HIGHEST APPLICATION PUBLICATION NUMBER: US2007006355
CA INDEXING IS CURRENT THROUGH 4 Jan 2007 (20070104/UPCA)
ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 4 Jan 2007 (20070104/PD)
REVISED CLASS FIELDS (/NCL) LAST RELOADED: Jun 2006
USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Jun 2006


=> s (inclusion bodies/clm)
           7350 INCLUSION/CLM
          31019 BODIES/CLM
L28         187 (INCLUSION BODIES/CLM)
                  ((INCLUSION(W)BODIES)/CLM)

=> s l28 and ay<2002
        3511059 AY<2002
L29          98 L28 AND AY<2002

=> s l29 and (multiple cop?/clm or multiple joined genes/clm)
          178138 MULTIPLE/CLM
          272782 COP?/CLM
            1075 MULTIPLE COP?/CLM
                   ((MULTIPLE(W)COP?)/CLM)
          178138 MULTIPLE/CLM
          118739 JOINED/CLM
            7551 GENES/CLM
               0 MULTIPLE JOINED GENES/CLM
                   ((MULTIPLE(W)JOINED(W)GENES)/CLM)
L30            2 L29 AND (MULTIPLE COP?/CLM OR MULTIPLE JOINED GENES/CLM)

=> d l30,cbib,clm,1-2

L30  ANSWER 1 OF 2  USPATFULL on STN
2001:25645 Recombinant bone morphogenetic protein heterodimers, compositions
    and methods of use.
    Israel, David, Concord, MA, United States
    Wolfman, Neil M., Dover, MA, United States
    Genetics Institute, Cambridge, MA, United States (U.S. corporation)
    US 6190880 B1 20010220
    APPLICATION: US 1995-469411  19950606 (8)
    PRIORITY: WO 1992-US9430  19921102
    DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM    What is claimed is:
       1. A method for producing a heterodimeric protein having bone
       stimulating activity comprising culturing under suitable conditions to
       produce said protein a selected host cell containing a first DNA
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sequence encoding a BMP selected from the group consisting of BMP-2 and BMP-4 and a second DNA sequence encoding BMP-5, said sequences each being under the control of a suitable regulatory sequence capable of directing co-expression of said proteins, and isolating said heterodimeric protein from the culture medium.

2. The method according to claim 1 wherein said first DNA is present on a first vector transfected into said host cell and said second DNA is present on a second vector transfected into said host cell.

3. The method according to claim 1 wherein both said BMPs are integrated into a chromosome of said host cell.

4. The method according to claim 1 wherein both BMPs are present on a single vector.

5. The method according to claim 2 wherein more than a single copy of the gene encoding each said BMP is present on each vector.

6. The method according to claim 1 wherein said host cell is a hybrid cell prepared by culturing two fused selected, stable host cells, each host cell transfected with a DNA sequence encoding said selected first or second BMP, said sequences under the control of a suitable regulatory sequence capable of directing expression of each protein, and isolating said heterodimer protein from the culture medium.

7. The method according to claim 1 wherein said host cell is a mammalian cell.

8. The method according to claim 1 wherein said host cell is an insect cell.

9. The method according to claim 1 wherein said host cell is a yeast cell.

10. A method for producing a heterodimeric protein having bone stimulating activity in a bacterial cell comprising culturing a selected host cell containing a first DNA sequence encoding a BMP selected from the group consisting of BMP-2 and BMP-4 under the control of a suitable regulatory sequence capable of directing expression of the protein under conditions suitable for the formation of a soluble, monomeric protein; isolating said monomeric protein from **inclusion bodies** and/or said culture medium; culturing a selected host cell containing a second DNA sequence encoding BMP-5 under the control of a suitable regulatory sequence capable of directing expression of the protein or protein fragment under said conditions to form a second soluble, monomeric protein; isolating said monomeric protein from **inclusion bodies** and/or said culture medium; and mixing said soluble monomeric proteins under conditions permitting the formation of dimeric proteins associated by at least one covalent disulfide bond; isolating from the mixture a heterodimeric protein.

11. The method according to claim 10 wherein said host cell is E. coli.

12. The method according to claim 10 wherein said conditions comprise treating said protein with a solubilizing agent.

13. An isolated recombinant heterodimeric protein having bone stimulating activity comprising a first BMP protein selected from the group consisting of BMP-2 and BMP-4 associated by at least one disulfide bond with a BMP-5 protein.

14. The protein according to claim 13 wherein said first protein is BMP-2.

15. The protein according to claim 13 wherein said first protein is BMP-4.

16. An isolated protein comprising a recombinant heterodimeric protein having bone stimulating activity produced by co-expression in a selected host cell and isolation from the culture medium said protein comprising a subunit of a first BMP selected from the group consisting of BMP-2 and BMP-4, associated by at least one disulfide bond with a second subunit of a BMP-5.

17. A cell line comprising a nucleotide sequence encoding a first BMP selected from the group consisting of BMP-2 and BMP-4 under control of a suitable expression regulatory system and a nucleotide sequence encoding a BMP-5 under control of a suitable expression regulatory system, said regulatory systems capable of directing the co-expression of said BMPs and the formation of heterodimeric protein.

18. The cell line according to claim 17 wherein said nucleotide sequences encoding BMP proteins are present in a single DNA molecule.

19. The cell line according to claim 17 wherein said nucleotide sequence encoding said first BMP is present on a first DNA molecule and said nucleotide sequence encoding said second BMP is present on a second DNA molecule.

20. The cell line according to claim 18 wherein said single DNA molecule comprises a first transcription unit containing a nucleic acid encoding said first BMP and a second transcription unit containing a nucleic acid encoding said second BMP.

21. The cell line according to claim 18 wherein said single DNA molecule comprises a single transcription unit containing **multiple copies** of said nucleic acid encoding said first BMP and **multiple copies** of said nucleic acid encoding said second BMP.

22. A DNA molecule comprising a sequence encoding a first BMP selected from the group consisting of BMP-2 and BMP4 and a sequence encoding a BMP-5 said sequences under the control of at least one suitable regulatory sequence capable of directing co-expression of each BMP.

23. The molecule according to claim 22 comprising a first transcription unit containing a nucleic acid encoding said first BMP and said second transcription unit containing a nucleic acid encoding said second BMP.

24. The molecule according to claim 22 comprising a single transcription unit containing **multiple copies** of said nucleic acid encoding said first BMP and **multiple copies** of said nucleic acid encoding said second BMP.

L30 ANSWER 2 OF 2 USPATFULL on STN
1999:15721 Recombinant bone morphogenetic protein heterodimers.
Israel, David, Concord, MA, United States
Wolfman, Neil M., Dover, MA, United States
Genetics Institute, Inc., Cambridge, MA, United States (U.S. corporation)
US 5866364 19990202
APPLICATION: US 1992-989847 19921127 (7)
PRIORITY: WO 1992-US9430 19921102
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A method for producing a heterodimeric protein having bone stimulating activity comprising (a) culturing under suitable conditions to produce said protein a selected host cell containing a first DNA sequence encoding BMP-2 and a second DNA sequence encoding BMP-6 said sequences each being under the control of a suitable regulatory sequence capable of directing co-expression of said proteins; and (b) isolating said heterodimeric protein from the culture medium.

2. The method according to claim 1 wherein said BMP-2 sequence is present on a first vector transfected into said host cell and said BMP-6 sequence is present on a second vector transfected into said host cell.

3. The method according to claim 2 wherein more than a single copy of the gene encoding each said BMP is present on each vector.

4. The method according to claim 1 wherein both said BMP sequences are integrated into a chromosome of said host cell.

5. The method according to claim 1 wherein both BMP sequences are present on a single vector.

6. The method according to claim 1 wherein said host cell is a mammalian cell.

7. The method according to claim 1 wherein said host cell is a yeast cell.

8. A method for producing a heterodimeric protein having bone stimulating activity comprising (a) culturing under suitable conditions to produce said protein a selected host cell containing a first BMP DNA sequence encoding a BMP selected from the group consisting of BMP-2 and BMP-4 and a second BMP DNA sequence encoding BMP-6 wherein said host cell is a hybrid cell prepared by culturing two fused selected, stable host cells, each host cell transfected with a DNA sequence encoding said selected first or second BMP, said sequences under the control of a suitable regulatory sequence capable of directing expression of each protein; and (b) isolating said heterodimer protein from the culture medium.

9. A method for producing heterodimeric protein having bone stimulating activity in a bacterial cell comprising (a) culturing a selected host cell containing a DNA sequence encoding BMP-2 under the control of a suitable regulatory sequence capable of directing expression of the protein under conditions suitable for the formation of a soluble, monomeric protein isolating said monomeric protein from **inclusion bodies** and/or said culture medium; (b) culturing a selected host cell containing a second DNA sequence encoding BMP-6 under the control of a suitable regulatory sequence capable of directing expression of the protein or protein fragment under said conditions to form a second soluble, monomeric protein; (c) isolating said monomeric protein from **inclusion bodies** and/or said culture medium; (d) mixing said soluble monomeric proteins under conditions permitting the formation of dimeric proteins associated by at least one covalent disulfide bond; and (e) isolating from the mixture a heterodimeric protein.

10. The method according to claim 9 wherein said host cell is E. coli.

11. The method according to claim 9 wherein said conditions comprise treating said protein with a solubilizing agent.

12. An isolated recombinant heterodimeric protein having bone stimulating activity comprising a BMP-2 protein subunit associated by at least one disulfide bond with a BMP-6 protein subunit.

13. A cell line comprising a nucleotide sequence encoding BMP-2 under control of a suitable expression regulatory system and a second nucleotide sequence encoding BMP-6 under control of a suitable expression regulatory system, said regulatory systems capable of directing the co-expression of said BMPs and the formation of heterodimeric protein.

14. The cell line according to claim 13 wherein said nucleotide sequences encoding said BMP-2 and BMP-6 proteins are present in a single DNA molecule.

15. The cell line according to claim 14 wherein said single DNA molecule comprises a first transcription unit containing a gene encoding said BMP-2 and a second transcription unit containing a gene encoding said BMP-6.

16. The cell line according to claim 14 wherein said single DNA molecule comprises a single transcription unit containing **multiple copies** of said gene encoding said BMP-2 and **multiple copies** of said gene encoding said BMP-6.

17. The cell line according to claim 13 wherein said nucleotide sequence encoding said BMP-2 is present on a first DNA molecule and said nucleotide sequence encoding said BMP-6 is present on a second DNA molecule.

18. A DNA molecule comprising a sequence encoding BMP-2 and a second sequence encoding BMP-6 said sequences under the control of at least one suitable regulatory sequence capable of directing co-expression of each BMP.

19. The molecule according to claim 18 comprising a first transcription unit containing a gene encoding said BMP-2 and said second transcription unit containing a gene encoding BMP-6.

20. The molecule according to claim 18 comprising a single transcription unit containing **multiple copies** of said gene encoding said BMP-2 and **multiple copies** of said gene encoding said BMP-6.

```
=> s (T1357 or T-1357)
             8 T1357
       1197426 T
          8823 1357
             8 T-1357
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=> s l31 and ay<2003
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L32           9 L31 AND AY<2003

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        143420 T/CLM
           229 1357/CLM
             0 T-1357/CLM
                 ((T(W)1357)/CLM)
             0 T1357/CLM
L33           0 L32 AND (T-1357/CLM OR T1357/CLM)

=> d l32,cbib,1-9

L32  ANSWER 1 OF 9  USPATFULL on STN
2006:227911 Transporter and ion channels.
    Baughn, Mariah R., 8547 W HORNER STREET, LOS ANGELES, CA, UNITED STATES
    90035
    Elliott, Vicki S., San Jose, CA, UNITED STATES
    Hafalia, April J A, Daly City, CA, UNITED STATES
    Yang, Junming, San Jose, CA, UNITED STATES
    Chawla, Narinder K., Union City, CA, UNITED STATES
    Ramkumar, Jayalaxmi, Fremont, CA, UNITED STATES
    Forsythe, Ian J., Edmonton, CA, UNITED STATES
    Lu, Yan, Mountain View, CA, UNITED STATES
    Tang, Y Tom, San Jose, CA, UNITED STATES
    Yue, Henry, Sunnyvale, CA, UNITED STATES
    Raumann, Brigitte E., Chicago, IL, UNITED STATES
    Lal, Preeti G., Santa Clara, CA, UNITED STATES
    Azimzai, Yalda, Oakland, CA, UNITED STATES
    Lu, Dyung Aina M., San Jose, CA, UNITED STATES
    Gandhi, Ameena R., San Frnacisco, CA, UNITED STATES
    Thornton, Michael B., Oakland, CA, UNITED STATES
    Nguyen, Danniel B., San Jose, CA, UNITED STATES
    Arvizu, Chandra S., San Diego, CA, UNITED STATES
    Emerling, Brooke M., Chicago, IL, UNITED STATES
    Swarnakar, Anita, San Francisco, CA, UNITED STATES
    Yao, Monique G., Mountain View, CA, UNITED STATES
    Ding, Li, Creve Coeur, MO, UNITED STATES
    He, Ann, San Jose, CA, UNITED STATES
    Griffin, Jennifer A., Fremont, CA, UNITED STATES
    Sanjanwala, Madhusudan M., Los Altos, CA, UNITED STATES
    Gietzen, Kimberly J., San Jose, CA, UNITED STATES
    Lee, Ernestine A., Kensington, CA, UNITED STATES
    Xu, Yuming, Mountain View, CA, UNITED STATES
    Au-Young, Janice K., Brisbane, CA, UNITED STATES
    Das, Debopriya, Oyster Bay, NY, UNITED STATES
    Lee, Soo Yeun, Mountain View, CA, UNITED STATES
    Chang, Hsin-Ru, Belmont, CA, UNITED STATES
    INCYTE CORPORATION, PALO ALTO, CA, UNITED STATES, 94304 (U.S. corporation)
    US 2006194275 A1 20060831
    APPLICATION: US 2002-474894  A1  20020412 (10)
    WO 2002-US11760 20020412 20040625  PCT 371 date
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L32  ANSWER 2 OF 9  USPATFULL on STN
2004:196788 Nucleic acid-associated proteins.
    Henry, Yue, Sunnyvale, CA, UNITED STATES
    Ding, Li, China, CHINA
    Baughn, Mariah R, Los Angeles, CA, UNITED STATES
```

Lal, Preeti G, Santa Clara, CA, UNITED STATES
Yue, Huibin, Cupertino, CA, UNITED STATES
Hafalia, April J A, Daly City, CA, UNITED STATES
Lee, Ernestine A, Kensington, CA, UNITED STATES
Ison, Craig H, San Jose, CA, UNITED STATES
Becha, Shanya D, San Francisco, CA, UNITED STATES
Gururajan, Rajagopal, San Jose, CA, UNITED STATES
Emerling, Brooke M, Chicago, IL, UNITED STATES
Griffin, Jennifer A, Fremont, CA, UNITED STATES
Tang, Y Tom, San Jose, CA, UNITED STATES
Lu, Dyung Aina M, San Jose, CA, UNITED STATES
Yao, Monique G, Mountain View, CA, UNITED STATES
Chawla, Narinder K, Union City, CA, UNITED STATES
Ramkumar, Jayalaxmi, Fremont, CA, UNITED STATES
Gandhi, Ameena R, San Francisco, CA, UNITED STATES
Lee, Soo Yeun, Mountain View, CA, UNITED STATES
Thomas, Richardson W, Redwood City, CA, UNITED STATES
Yang, Junming, San Jose, CA, UNITED STATES
Elliott, Vicki S, San Jose, CA, UNITED STATES
Lu, Yan, Mountain View, CA, UNITED STATES
Thangavelu, Kavitha, Sunnyvale, CA, UNITED STATES
He, Ann, San Jose, CA, UNITED STATES
Azimzai, Yalda, Oakland, CA, UNITED STATES
Raumann, Brigitte E, Chicago, IL, UNITED STATES
Swarnakar, Anita, San Francisco, CA, UNITED STATES
Burford, Neil, Durham, CT, UNITED STATES
US 2004152093 A1 20040805
APPLICATION: US 2003-476924 A1 20031104 (10)
WO 2002-US14276 20020502
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L32 ANSWER 3 OF 9 USPATFULL on STN
2004:140285 Glucan chain length domains.
Commuri, Padma, Ankeny, IA, UNITED STATES
Keeling, Peter L., Ames, IA, UNITED STATES
Ramirez, Nona, Ames, IA, UNITED STATES
McKean, Angela, Ames, IA, UNITED STATES
Gao, Zhong, Ames, IA, UNITED STATES
Guan, Hanping, Ames, IA, UNITED STATES
US 2004107461 A1 20040603
APPLICATION: US 2002-109048 A1 20020329 (10)
PRIORITY: US 2001-279720P 20010330 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L32 ANSWER 4 OF 9 USPATFULL on STN
2004:114927 Molecules for disease detection and treatment.
Lal, Preeti G, Santa Clara, CA, UNITED STATES
Baughn, Mariah R, Los Angeles, CA, UNITED STATES
Yao, Monique G, Mountain View, CA, UNITED STATES
Chawla, Narinder K, Union City, CA, UNITED STATES
Elliott, Vicki S, San Jose, CA, UNITED STATES
Xu, Yuming, Mountain View, CA, UNITED STATES
Honchell, Cynthia D, San Carlos, CA, UNITED STATES
Yue, Henry, Sunnyvale, CA, UNITED STATES
Ding, Li, Creve Couer, MO, UNITED STATES
Gietzen, Kimberly J, San Jose, CA, UNITED STATES
Ison, Craig H, San Jose, CA, UNITED STATES
Lu, Dyung Aina M, San Jose, CA, UNITED STATES
Hafalia, April JA, Daly City, CA, UNITED STATES
Gandhi, Ameena R, San Francisco, CA, UNITED STATES
Thangavelu, Kavitha, Sunnyvale, CA, UNITED STATES
Sanjanwala, Madhusudan M, Los Altos, CA, UNITED STATES
Tang, Y Tom, San Jose, CA, UNITED STATES
Ramkumar, Jayalaxmi, Fremont, CA, UNITED STATES
Griffin, Jennifer A, Fremont, CA, UNITED STATES
Swarnakar, Anita, San Francisco, CA, UNITED STATES
Azimzai, Yalda, Oakland, CA, UNITED STATES
Sapperstein, Stephanie K, Redwood City, CA, UNITED STATES
Burford, Neil, Durham, CT, UNITED STATES
Lee, Ernestine A, Castro Valley, CA, UNITED STATES
Lu, Yan, Mountain View, CA, UNITED STATES
Tran, Uyen K, San Jose, CA, UNITED STATES
Marquis, Joseph P, San Jos, CA, UNITED STATES

US 2004087773 A1 20040506
APPLICATION: US 2003-467433 A1 20030806 (10)
WO 2002-US3709 20020208
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L32 ANSWER 5 OF 9 USPATFULL on STN
2003:231989 Polynucleotide encoding a novel TRP channel family member, LTRPC3, and splice variants thereof.
Lee, Ning, Belle Mead, NJ, UNITED STATES
Chen, Jian, Princeton, NJ, UNITED STATES
Feder, John, Belle Mead, NJ, UNITED STATES
Wu, Shujian, Langhorne, PA, UNITED STATES
Lee, Liana, North Brunswick, NJ, UNITED STATES
Blanar, Michael A., Malvern, PA, UNITED STATES
Bol, David, Langhorne, PA, UNITED STATES
Levesque, Paul C., Yardley, PA, UNITED STATES
Sun, Lucy, Newtown, PA, UNITED STATES
US 2003162189 A1 20030828
APPLICATION: US 2002-210152 A1 20020801 (10)
PRIORITY: US 2001-309544P 20010802 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L32 ANSWER 6 OF 9 USPATFULL on STN
2003:225676 Genetic analysis systems and methods.
Cox, David R., Belmont, CA, UNITED STATES
Margus, Bradley A., Boca Raton, FL, UNITED STATES
Perlegen Sciences, Inc., Mountain View, CA (U.S. corporation)
US 2003157488 A1 20030821
APPLICATION: US 2002-42819 A1 20020107 (10)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L32 ANSWER 7 OF 9 USPATFULL on STN
2003:152915 Process for making antifusogenic fusion peptides that form inclusion bodies.
Hoess, Eva, Muenchen, GERMANY, FEDERAL REPUBLIC OF
Meier, Thomas, Muenchen, GERMANY, FEDERAL REPUBLIC OF
Pestlin, Gabriele, Muenchen, GERMANY, FEDERAL REPUBLIC OF
Popp, Friedrich, Penzberg, GERMANY, FEDERAL REPUBLIC OF
Reichert, Klaus, Weilheim, GERMANY, FEDERAL REPUBLIC OF
Schmuck, Rainer, Benediktbeuern, GERMANY, FEDERAL REPUBLIC OF
Schneidinger, Bernd, Hohenschaeftlarn/Neufahrn, GERMANY, FEDERAL REPUBLIC OF
Seidel, Christoph, Weilheim, GERMANY, FEDERAL REPUBLIC OF
Tischer, Wilhelm, Peissenberg, GERMANY, FEDERAL REPUBLIC OF
US 2003104581 A1 20030605
APPLICATION: US 2002-158742 A1 20020530 (10)
PRIORITY: EP 2001-114497 20010615
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L32 ANSWER 8 OF 9 USPATFULL on STN
94:68435 Ni-Ti-Al alloys.
Cahn, Robert W., Cambridge, England
Rolls-Royce plc, London, England (non-U.S. corporation)
US 5336340 19940809
APPLICATION: US 1993-39494 19930430 (8)
PRIORITY: GB 1990-25486 19901123
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L32 ANSWER 9 OF 9 USPATFULL on STN
84:42777 Polyether polyurethane elastomers.
Kibler, Richard W., Cuyahoga Falls, OH, United States
The Firestone Tire & Rubber Company, Akron, OH, United States (U.S. corporation)
US 4463155 19840731
APPLICATION: US 1982-377742 19820513 (6)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

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        10424 1249
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E7            1      BOLOGNESI GUALTIERO/IN
E8            2      BOLOGNESI MARIA L/IN
E9            1      BOLOGNESI MARIA LAURA/IN
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E5            2      LAMBERT DAMIEN/IN
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E7            2      LAMBERT DANIEL J/IN
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E9            2      LAMBERT DARYL G/IN
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E12           5      LAMBERT DAVID/IN

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E3            6 --> LAMBERT DENNIS M/IN
E4           16      LAMBERT DENNIS MICHAEL/IN
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E6            2      LAMBERT DEREK/IN
E7            1      LAMBERT DEREK W/IN
E8           12      LAMBERT DIANE/IN
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E12           1      LAMBERT DON/IN

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L37          22 "LAMBERT DENNIS M"/IN OR "LAMBERT DENNIS MICHAEL"/IN

=> d his

     (FILE 'HOME' ENTERED AT 11:43:32 ON 08 JAN 2007)

     FILE 'USPATFULL' ENTERED AT 11:43:44 ON 08 JAN 2007
                E KACZMAREK ALEXANDRA/IN
L1              1 S E3
                E KOPETZKI ERHARD/IN
L2             26 S E3
L3             16 S L2 AND (INCLUSION BODIES)
L4              3 S L3 AND (INCLUSION BODIES/CLM)
L5              2 S L4 NOT L1
L6             13 S L3 NOT L4
L7             13 S L6 AND AY<2003
                E SCHANTZ CHRISTIAN/IN
L8              2 S E3
L9              0 S L8 NOT (L1 OR L2)
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L10           6 S E3
L11           5 S L10 NOT (L1 OR L2)

     FILE 'WPIDS' ENTERED AT 11:49:15 ON 08 JAN 2007
                E KACZMAREK A/IN
L12          15 S E3
L13           1 S L12 AND (INCLUSION BODIES)
                E KOPETZKI E/IN
L14          30 S E3
L15           9 S L14 AND (INCLUSION BODIES)
L16           8 S L15 NOT L12
                E SCHANTZ C/IN
L17           5 S E3
                E SEEBER S/IN
L18          19 S E3
L19          18 S L18 NOT (L12 OR L14)
L20           2 S L19 AND (INCLUSION BODIES)

     FILE 'MEDLINE' ENTERED AT 11:53:20 ON 08 JAN 2007
                E KACZMAREK A/AU
L21          49 S E3
L22           0 S L21 AND (INCLUSION BODIES)
                E KOPETZKI E/AU
L23          19 S E3-E4
L24           0 S L23 AND (INCLUSION BODIES)
                E SCHANTZ C/AU
L25           2 S E3
                E SEEBER S/AU
L26         329 S E3
L27           0 S L26 AND (INCLUSION BODIES)

     FILE 'USPATFULL' ENTERED AT 11:55:20 ON 08 JAN 2007
L28         187 S (INCLUSION BODIES/CLM)
L29          98 S L28 AND AY<2002
L30           2 S L29 AND (MULTIPLE COP?/CLM OR MULTIPLE JOINED GENES/CLM)
L31          13 S (T1357 OR T-1357)
L32           9 S L31 AND AY<2003
L33           0 S L32 AND (T-1357/CLM OR T1357/CLM)
L34         458 S T-1249
                E BOLOGNESI D P/IN
L35          12 S E4
L36           0 S L35 AND L34
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                E LAMBERT DENNIS M/IN
L37          22 S E3 OR E4

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L38           1 L37 AND L34

=> d l38,cbib,ab

L38  ANSWER 1 OF 1  USPATFULL on STN
2003:129917 Hybrid polypeptides with enhanced pharmacokinetic properties.
    Barney, Shawn, Apex, NC, United States
    Guthrie, Kelly I., Graham, NC, United States
    Merutka, Gene, Hillsborough, NC, United States
    Anwer, Mohmed K., Foster City, CA, United States
    Lambert, Dennis M., Cary, NC, United States
    Trimeris, Inc., Durham, NC, United States (U.S. corporation)
    US 6562787 B1 20030513
    APPLICATION: US 2001-834784  20010413 (9)
    DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB     The present invention relates to enhancer peptide sequences originally
       derived from various retroviral envelope (gp41) protein sequences that
       enhance the pharmacokinetic properties of any core polypeptide to which
       they are linked. The invention is based on the discovery that hybrid
       polypeptides comprising the enhancer peptide sequences linked to a core
       polypeptide possess enhanced pharmacokinetic properties such as
       increased half life. The invention further relates to methods for
       enhancing the pharmacokinetic properties of any core polypeptide through
       linkage of the enhancer peptide sequences to the core polypeptide. The
       core polypeptides to be used in the practice of the invention can
       include any pharmacologically useful peptide that can be used, for
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example, as a therapeutic or prophylactic reagent.

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=> e barney s/in
E1            1     BARNEY RONALD W/IN
E2            1     BARNEY RUSSELL T/IN
E3            0 --> BARNEY S/IN
E4            2     BARNEY SANTIAGO H/IN
E5            2     BARNEY SCOTT/IN
E6            1     BARNEY SHANE R/IN
E7            9     BARNEY SHAWN/IN
E8            1     BARNEY SHAWN O APOS LIN/IN
E9           12     BARNEY SHAWN O LIN/IN
E10           1     BARNEY TANIS M/IN
E11           3     BARNEY TIMOTHY A/IN
E12           2     BARNEY TRAVIS L/IN

=> s e7-e9
              9 "BARNEY SHAWN"/IN
              1 "BARNEY SHAWN O APOS LIN"/IN
             12 "BARNEY SHAWN O LIN"/IN
L39          22 ("BARNEY SHAWN"/IN OR "BARNEY SHAWN O APOS LIN"/IN OR "BARNEY
                SHAWN O LIN"/IN)

=> d his

     (FILE 'HOME' ENTERED AT 11:43:32 ON 08 JAN 2007)

     FILE 'USPATFULL' ENTERED AT 11:43:44 ON 08 JAN 2007
                E KACZMAREK ALEXANDRA/IN
L1            1 S E3
                E KOPETZKI ERHARD/IN
L2           26 S E3
L3           16 S L2 AND (INCLUSION BODIES)
L4            3 S L3 AND (INCLUSION BODIES/CLM)
L5            2 S L4 NOT L1
L6           13 S L3 NOT L4
L7           13 S L6 AND AY<2003
                E SCHANTZ CHRISTIAN/IN
L8            2 S E3
L9            0 S L8 NOT (L1 OR L2)
                E SEEBER STEFAN/IN
L10           6 S E3
L11           5 S L10 NOT (L1 OR L2)

     FILE 'WPIDS' ENTERED AT 11:49:15 ON 08 JAN 2007
                E KACZMAREK A/IN
L12          15 S E3
L13           1 S L12 AND (INCLUSION BODIES)
                E KOPETZKI E/IN
L14          30 S E3
L15           9 S L14 AND (INCLUSION BODIES)
L16           8 S L15 NOT L12
                E SCHANTZ C/IN
L17           5 S E3
                E SEEBER S/IN
L18          19 S E3
L19          18 S L18 NOT (L12 OR L14)
L20           2 S L19 AND (INCLUSION BODIES)

     FILE 'MEDLINE' ENTERED AT 11:53:20 ON 08 JAN 2007
                E KACZMAREK A/AU
L21          49 S E3
L22           0 S L21 AND (INCLUSION BODIES)
                E KOPETZKI E/AU
L23          19 S E3-E4
L24           0 S L23 AND (INCLUSION BODIES)
                E SCHANTZ C/AU
L25           2 S E3
                E SEEBER S/AU
L26         329 S E3
L27           0 S L26 AND (INCLUSION BODIES)

     FILE 'USPATFULL' ENTERED AT 11:55:20 ON 08 JAN 2007
L28         187 S (INCLUSION BODIES/CLM)
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L29           98 S L28 AND AY<2002
L30            2 S L29 AND (MULTIPLE COP?/CLM OR MULTIPLE JOINED GENES/CLM)
L31           13 S (T1357 OR T-1357)
L32            9 S L31 AND AY<2003
L33            0 S L32 AND (T-1357/CLM OR T1357/CLM)
L34          458 S T-1249
                 E BOLOGNESI D P/IN
L35           12 S E4
L36            0 S L35 AND L34
                 E LAMBERT D M/IN
                 E LAMBERT DENNIS M/IN
L37           22 S E3 OR E4
L38            1 S L37 AND L34
                 E BARNEY S/IN
L39           22 S E7-E9

=> s l39 and l34
L40            1 L39 AND L34

=> d l40,cbib

L40  ANSWER 1 OF 1  USPATFULL on STN
2003:129917 Hybrid polypeptides with enhanced pharmacokinetic properties.
    Barney, Shawn, Apex, NC, United States
    Guthrie, Kelly I., Graham, NC, United States
    Merutka, Gene, Hillsborough, NC, United States
    Anwer, Mohmed K., Foster City, CA, United States
    Lambert, Dennis M., Cary, NC, United States
    Trimeris, Inc., Durham, NC, United States (U.S. corporation)
    US 6562787 B1 20030513
    APPLICATION: US 2001-834784  20010413 (9)
    DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.


=> file medline
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                      39.52     135.01

FILE 'MEDLINE' ENTERED AT 12:05:13 ON 08 JAN 2007

 FILE LAST UPDATED: 6 Jan 2007 (20070106/UP).  FILE COVERS 1950 TO DATE.

  All regular MEDLINE updates from November 15 to December 16 have been
  added to MEDLINE, along with 2007 Medical Subject Headings (MeSH(R))
  and 2007 tree numbers.

  The annual reload will be available in early 2007.

  This file contains CAS Registry Numbers for easy and accurate
  substance identification.

=> s (inclusion bodies0
UNMATCHED LEFT PARENTHESIS '(INCLUSION'
The number of right parentheses in a query must be equal to the
number of left parentheses.

=> s (inclusion bodies)
         66032 INCLUSION
        109790 BODIES
L41      17324 (INCLUSION BODIES)
                 (INCLUSION(W)BODIES)

=> s l41 and purification
        597409 PURIFICATION
L42       2797 L41 AND PURIFICATION

=> s l42 and py<2002
      13447730 PY<2002
                 (PY<20020000)
L43       2197 L42 AND PY<2002

=> s l43 and (multicopy or multiple copies or multiple joined)
          3060 MULTICOPY
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        498907 MULTIPLE
         25703 COPIES
          2581 MULTIPLE COPIES
                 (MULTIPLE(W)COPIES)
        498907 MULTIPLE
          6837 JOINED
             7 MULTIPLE JOINED
                 (MULTIPLE(W)JOINED)
L44           2 L43 AND (MULTICOPY OR MULTIPLE COPIES OR MULTIPLE JOINED)

=> d l44,cbib,ab,1-2

L44  ANSWER 1 OF 2     MEDLINE on STN
1999364542.     PubMed ID: 10437831.   Isolation and characterization of cDNAs
     encoding mitochondrial phosphate transporters in soybean, maize, rice, and
     Arabidopis. Takabatake R; Hata S; Taniguchi M; Kouchi H; Sugiyama T; Izui
     K. (Division of Applied Biosciences, Graduate School of Agriculture, Kyoto
     University, Japan. ) Plant molecular biology, (1999 Jun) Vol. 40, No. 3,
     pp. 479-86.  Journal code: 9106343. ISSN: 0167-4412. Pub. country:
     Netherlands. Language: English.
AB   cDNA clones encoding mitochondrial phosphate transporters were isolated
     from four herbaceous plants.  The cDNAs for the soybean, maize and rice
     transporters contained entire coding regions, whereas the Arabidopsis cDNA
     lacked the 5' portion.  The hydropathy profiles of the deduced amino acid
     sequences predicted the existence of six membrane-spanning domains which
     are highly conserved in the mitochondrial transporter family.  In
     soybeans, the mRNA level for the transporter was high in tissues
     containing dividing cells.  It was suggested that there are multiple
     copies of transporter genes in both dicots and monocots.  The soybean
     transporter was expressed as inclusion bodies in Escherichia coli,
     solubilized with detergents, and then reconstituted into liposomes.  The
     resulting proteoliposomes exhibited high phosphate transport activity.
     The activity was inhibited by N-ethylmaleimide, like those of mammalian
     phosphate transporters.

L44  ANSWER 2 OF 2     MEDLINE on STN
94305408.     PubMed ID: 8032261.   Purification and characterization of the
     U-particle, a cellular constituent whose synthesis is stimulated by
     Mengovirus infection. Mulvey M R; Fang H; Scraba D G. (Department of
     Biochemistry, University of Alberta, Edmonton, Canada. ) Archives of
     virology. Supplementum, (1994) Vol. 9, pp. 299-306.  Journal code:
     9214275. ISSN: 0939-1983. Pub. country: Austria. Language: English.
AB   We have isolated a cellular protein particle whose synthesis is induced by
     infection with Mengovirus or TMEV.  The U-particle inhibits translation in
     vitro and binds to both capped and uncapped mRNA's.  It is spherical, 12
     nm in diameter, and is composed of multiple copies of two polypeptide
     subunits having molecular weights of 23,000 and 25,000 which do not appear
     to be glycosylated or phosphorylated.  U-particles are capable of
     inhibiting mRNA translation in vitro.


=> d his

     (FILE 'HOME' ENTERED AT 11:43:32 ON 08 JAN 2007)

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                E SEEBER STEFAN/IN
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                E LAMBERT DENNIS M/IN
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                E BARNEY S/IN
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L40           1 S L39 AND L34

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L45          18 L43 AND REVIEW?

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L45  ANSWER 1 OF 18     MEDLINE on STN
2002045601.    PubMed ID: 11757069.   Applications of novel affinity cassette
     methods: use of peptide fusion handles for the purification of
     recombinant proteins. Hearn M T; Acosta D. (Centre for Bioprocess
     Technology, Department of Biochemistry and Molecular Biology, Monash
     University, Wellington Road, Clayton 3800 Australia..
     milton.hearn@med.monash.edu.au) . Journal of molecular recognition : JMR,
     (2001 Nov-Dec) Vol. 14, No. 6, pp. 323-69.  Ref: 329. Journal code:
     9004580. ISSN: 0952-3499. Pub. country: England: United Kingdom. Language:
     English.
AB   In this article, recent progress related to the use of different types of
     polypeptide fusion handles or 'tags' for the purification of recombinant
     proteins are critically discussed.  In addition, novel aspects of the
     molecular cassette concept are elaborated, together with areas of
     potential application of these fundamental principles in molecular
     recognition.  As evident from this review, the use of these concepts
     provides a powerful strategy for the high throughput isolation and
     purification of recombinant proteins and their derived domains,
     generated from functional genomic or zeomic studies, as part of the
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bioprocess technology leading to their commercial development, and in the study of molecular recognition phenomena per se. In addition, similar concepts can be exploited for high sensitivity analysis and detection, for the characterisation of protein bait/prey interactions at the molecular level, and for the immobilisation and directed orientation of proteins for use as biocatalysts/biosensors.

L45 ANSWER 2 OF 18 MEDLINE on STN
1999324614. PubMed ID: 10396292. Cytomegalovirus-infected cells in routinely prepared peripheral blood films of immunosuppressed patients. Pooley R J Jr; Peterson L; Finn W G; Kroft S H. (Northwestern University Medical School, Chicago, IL, USA.) American journal of clinical pathology, **(1999 Jul)** Vol. 112, No. 1, pp. 108-12. Journal code: 0370470. ISSN: 0002-9173. Pub. country: United States. Language: English.
AB We describe 4 patients identified over 5 years with large atypical cells on the feathered edge of routinely prepared peripheral blood films. Films were **reviewed** either as part of a blood film consultation or a bone marrow examination. The cells were 50 to 60 microns in diameter, with granular eosinophilic cytoplasmic inclusions and eccentric enlarged nuclei. Additional studies including buffy coat preparations and immunohistochemistry revealed that these were circulating cytomegalovirus (CMV)-infected cells, most likely of endothelial origin. All patients were immunocompromised (3 had HIV infection, and 1 was an organ transplant recipient) and had clinical evidence of CMV infection. The unique appearance of these cells at Wright-Giemsa staining, and their possible misidentification as malignant cells or other cells, highlights the need for pathologists to be aware of their morphologic features and possible clinical implication.

L45 ANSWER 3 OF 18 MEDLINE on STN
97422857. PubMed ID: 9276922. Molecular chaperones, folding catalysts, and the recovery of active recombinant proteins from E. coli. To fold or to refold. Thomas J G; Ayling A; Baneyx F. (University of Washington, Department of Chemical Engineering, Seattle 98195-1750, USA.) Applied biochemistry and biotechnology, **(1997 Jun)** Vol. 66, No. 3, pp. 197-238. Ref: 214. Journal code: 8208561. ISSN: 0273-2289. Pub. country: United States. Language: English.
AB The high-level expression of recombinant gene products in the gram-negative bacterium Escherichia coli often results in the misfolding of the protein of interest and its subsequent degradation by cellular proteases or its deposition into biologically inactive aggregates known as **inclusion bodies**. It has recently become clear that in vivo protein folding is an energy-dependent process mediated by two classes of folding modulators. Molecular chaperones, such as the DnaK-DnaJ-GrpE and GroEL-GroES systems, suppress off-pathway aggregation reactions and facilitate proper folding through ATP-coordinated cycles of binding and release of folding intermediates. On the other hand, folding catalysts (foldases) accelerate rate-limiting steps along the protein folding pathway such as the cis/trans isomerization of peptidyl-prolyl bonds and the formation and reshuffling of disulfide bridges. Manipulating the cytoplasmic folding environment by increasing the intracellular concentration of all or specific folding modulators, or by inactivating genes encoding these proteins, holds great promise in facilitating the production and **purification** of heterologous proteins. Purified folding modulators and artificial systems that mimic their mode of action have also proven useful in improving the in vitro refolding yields of chemically denatured polypeptides. This **review** examines the usefulness and limitations of molecular chaperones and folding catalysts in both in vivo and in vitro folding processes.

L45 ANSWER 4 OF 18 MEDLINE on STN
97149198. PubMed ID: 8996006. Cytomegalovirus as a primary pulmonary pathogen in AIDS. Waxman A B; Goldie S J; Brett-Smith H; Matthay R A. (Department of Internal Medicine, Yale University School of Medicine, New Haven, Conn 06520-8057, USA.) Chest, **(1997 Jan)** Vol. 111, No. 1, pp. 128-34. Journal code: 0231335. ISSN: 0012-3692. Pub. country: United States. Language: English.
AB In patients with AIDS, isolation of cytomegalovirus (CMV) from respiratory secretions is common. It is often found with other pathogens, which has led to debate regarding its role as a primary pulmonary pathogen. A retrospective investigation of patients with AIDS and CMV as a sole pulmonary isolate was performed in an attempt to describe their clinical presentation and course. All patients admitted to the hospital with pneumonia and with BAL or transbronchial biopsy (TBB) specimen positive

for CMV between 1991 and 1994 were identified through a **review** of inpatient records. Inclusion criteria included positive CMV cultures from BAL, cytomegalic **inclusion bodies** from BAL or TBB, and thorough documentation of the absence of other pulmonary pathogens. Nine patients met the inclusion criteria for CMV pneumonitis. Seven were male and two were female, ages 26 to 44 years, and all had a history of opportunistic infections. Typical clinical presentation was characterized by increased respiratory rate, hypoxemia, and diffuse interstitial infiltrates. The mean CD4 count was 29.6 (+/- 22) cells per cubic millimeter, mean lactate dehydrogenase level was 414 (+/- 301) IU/L, and in seven patients in whom CMV antigen was measured it was greater than 50 positive cells per 200,000 WBCs. Three untreated patients died of respiratory failure and three had autopsy confirmation of CMV pneumonia. Five patients were treated with anti-CMV therapy for at least 2 weeks, and all demonstrated improvement in symptoms, oxygen saturation, and chest radiograph. At 3 months follow-up, all five patients were asymptomatic with no pulmonary symptoms. At 6 months follow-up, three of the five patients remained asymptomatic; the other two died of other opportunistic infections. In at least these nine patients, CMV represented a primary pulmonary pathogen. Patients who were treated responded quickly and were able to be discharged home from the hospital with marked improvement in their symptoms. We recommend that clinicians consider this diagnosis in the proper setting and consider treatment with anti-CMV therapy.

L45 ANSWER 5 OF 18 MEDLINE on STN

97093497. PubMed ID: 8939059. **Inclusion bodies** and **purification** of proteins in biologically active forms. Mukhopadhyay A. (National Institute of Immunology, Aruna Asaf Ali Marg, New Delhi, India.) Advances in biochemical engineering/biotechnology, **(1997)** Vol. 56, pp. 61-109. Ref: 160. Journal code: 8307733. ISSN: 0724-6145. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Even though recombinant DNA technology has made possible the production of valuable therapeutic proteins, its accumulation in the host cell as inclusion body poses serious problems in the recovery of functionally active proteins. In the last twenty years, alternative techniques have been evolved to purify biologically active proteins from **inclusion bodies**. Most of these remain only as inventions and very few are commercially exploited. This **review** summarizes the developments in isolation, refolding and **purification** of proteins from **inclusion bodies** that could be used for vaccine and non-vaccine applications. The second section involves a discussion on **inclusion bodies**, how they are formed, and their physicochemical properties. In vivo protein folding in Escherichia coli and kinetics of in vitro protein folding are the subjects of the third and fourth sections respectively. The next section covers the recovery of bioactive protein from **inclusion bodies**: it includes isolation of inclusion body from host cell debris, **purification** in denatured state alternate refolding techniques, and final **purification** of active molecules. Since purity and safety are two important issues in therapeutic grade proteins, the following three sections are devoted to immunological and biological characterization of biomolecules, nature, and type of impurities normally encountered, and their detection. Lastly, two case studies are discussed to demonstrate the sequence of process steps involved.

L45 ANSWER 6 OF 18 MEDLINE on STN

97042090. PubMed ID: 8887361. Protein folding in vivo and renaturation of recombinant proteins from **inclusion bodies**. Guise A D; West S M; Chaudhuri J B. (School of Chemical Engineering, University of Bath, UK.) Molecular biotechnology, **(1996 Aug)** Vol. 6, No. 1, pp. 53-64. Ref: 67. Journal code: 9423533. ISSN: 1073-6085. Pub. country: United States. Language: English.

AB Eukaryotic proteins expressed in Escherichia coli often accumulate within the cell as insoluble protein aggregates or **inclusion bodies**. The recovery of structure and activity from **inclusion bodies** is a complex process, there are no general rules for efficient renaturation. Research into understanding how proteins fold in vivo is giving rise to potentially new refolding methods, for example, using molecular chaperones. In this article we **review** what is understood about the main three classes of chaperone: the Stress 60, Stress 70, and Stress 90 proteins. We also give an overview of current process strategies for renaturing **inclusion bodies**, and report the use of novel developments that have enhanced refolding yields.

L45 ANSWER 7 OF 18 MEDLINE on STN

96075435. PubMed ID: 7493381. Targeting c-erbB-2 expressing tumors using

single-chain Fv monomers and dimers. Tai M S; McCartney J E; Adams G P; Jin D; Hudziak R M; Oppermann H; Laminet A A; Bookman M A; Wolf E J; Liu S; +. (Creative BioMolecules, Inc., Hopkinton, Massachusetts 01748, USA.) Cancer research, **(1995 Dec 1)** Vol. 55, No. 23 Suppl, pp. 5983s-5989s. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.

AB Single-chain Fv proteins containing a COOH-terminal cysteine (sFv') were constructed by using an antidigoxin 26.10 sFv and an anti-c-erbB-2 741F8 sFv. The fully active sFv' proteins were prepared by expression in Escherichia coli as insoluble **inclusion bodies**, followed by in vitro refolding using glutathione redox buffers and **purification**. The COOH-terminal cysteines of the refolded sFv' proteins were protected by a blocking group presumed to be the glutathionyl peptide, which was easily and selectively removed by gentle reduction. Air oxidation of the reduced sFv' monomers resulted in the efficient formation of disulfide-linked sFv' homodimers, designated (sFv')2, which were stable under oxidizing conditions and relatively slow to be disrupted under reducing conditions. The (26-10-1 sFv')-(741F8-1 sFv') heterodimer was prepared and possessed dual-antigen specificity; the active bispecific (sFv')2 dimerized under native conditions, apparently as a manifestation of self-association by the 741F8 sFv' subunit. Biodistribution and imaging studies that were performed on mice bearing human SK-OV-3 tumor xenografts that express the c-erbB-2 as a cell surface antigen were **reviewed**. Radioiodinated 741F8-2 (sFv')2 homodimer localized to the tumors with high specificity, as evidenced by excellent tumor:normal tissue ratios. Sagittal section autoradiography of whole animals 24 h after administration of antibody species revealed that 741F8 (sFv')2 produced a stronger tumor image than comparable doses of the 741F8 Fab, monomeric sFv', and the 26-10 (sFv')2 control without the high nonspecific background distribution of the 741F8 IgG.

L45 ANSWER 8 OF 18 MEDLINE on STN

94121850. PubMed ID: 8292306. Viral association with Crohn's disease. Smith M S; Wakefield A J. (Inflammatory Bowel Disease Study Group, Royal Free Hospital School of Medicine, London, UK.) Annals of medicine, **(1993 Dec)** Vol. 25, No. 6, pp. 557-61. Ref: 56. Journal code: 8906388. ISSN: 0785-3890. Pub. country: Finland. Language: English.

AB Crohn's disease is an inflammatory disorder of the gastrointestinal tract, the cause of which remains unknown. Since the first description by Dalziel in 1913 (1), similarities between Crohn's disease and intestinal mycobacterial infection, particularly Johne's disease in ruminants, have been widely recognized (2, 3). After Mitchell and Rees demonstrated the transmission of granulomata from Crohn's disease by injecting intestinal homogenates into the footpads of mice (4), there followed many studies attempting to identify infective agents within the bowel of patients with Crohn's disease. Although Mycobacterium paratuberculosis has been identified in intestinal tissue from a proportion of patients with Crohn's disease, a convincing role for this agent in the aetiology of Crohn's disease has not been established (5). Likewise, extensive studies into bacterial (6-9) and viral (10) agents potentially associated with Crohn's disease have been inconclusive, although recent ultrastructural observations of viral particles within submucosal granulomata have added a new impetus to the search (11, 12). This **review** examines the evidence for an association between Crohn's disease and viral infection from epidemiological studies, transmission and cell culture, specific immunological responses, ultrastructure and from molecular biological techniques.

L45 ANSWER 9 OF 18 MEDLINE on STN

93235201. PubMed ID: 8386399. [Equine granulocytic ehrlichiosis (EGE), a **review**]. Equine granulocytaire ehrlichiosis (EGE), een overzicht. van der Kolk J H; van der Wijden M W; Jongejan F. (Vakgroep Inwendige Zieketen en Voeding der Grote Huisdieren, Faculteit Diergeneeskunde, Utrecht.) Tijdschrift voor diergeneeskunde, **(1993 Apr 1)** Vol. 118, No. 7, pp. 227-9. Ref: 30. Journal code: 0031550. ISSN: 0040-7453. Pub. country: Netherlands. Language: Dutch.

AB Equine granulocytic ehrlichiosis (EGE) has been observed in the U.S.A., Brazil, Germany, Sweden, Switzerland and possibly in Great Britain. The causative agent is rickettsia Ehrlichia equi, identified for the first time in 1969. The clinical features of the disease are anorexia, fever, depression, (limb) oedema, icterus, ataxia, petechiae and orchitis. Hematologic changes are leukopenia, thrombocytopenia, anemia and cytoplasmic **inclusion bodies** in the neutrophils and eosinophils. Vasculitis may be observed at autopsy. Following a positive hematological diagnosis (Giemsa stained blood smear) of EGE, treatment with

oxytetracycline can be initiated.

L45 ANSWER 10 OF 18 MEDLINE on STN
93125969. PubMed ID: 8380493. Cytomegalovirus infections of the oral cavity. A report of six cases and **review** of the literature. Jones A C; Freedman P D; Phelan J A; Baughman R A; Kerpel S M. (Department of Oral Diagnostic Sciences, University of Florida College of Dentistry, Gainesville.) Oral surgery, oral medicine, and oral pathology, **(1993 Jan)** Vol. 75, No. 1, pp. 76-85. Ref: 34. Journal code: 0376406. ISSN: 0030-4220. Pub. country: United States. Language: English.
AB Cytomegalovirus is responsible for a significant percentage of asymptomatic viral infections worldwide. Although virtually any cell or organ may be infected, involvement of the oral cavity is uncommon. Only nine well-documented examples of intraoral cytomegalovirus infections were found in a **review** of the English-language literature. We report six additional examples, five of which possessed unique clinical and histopathologic characteristics. The light and electron-microscopic features of cytomegalovirus are discussed, as well as diagnostic adjuncts such as immunohistochemistry and deoxyribonucleic acid in situ hybridization.

L45 ANSWER 11 OF 18 MEDLINE on STN
91325466. PubMed ID: 1650859. Necrotizing myelopathy associated with malignancy caused by herpes simplex virus type 2: clinical report of two cases and literature **review**. Nakagawa M; Nakamura A; Kubota R; Kakazu T; Kuba M; Nakasone K; Iwamasa T. (Department of Neurology, National Okinawa Hospital, Ginowan, Japan.) Japanese journal of medicine, **(1991 Mar-Apr)** Vol. 30, No. 2, pp. 182-8. Journal code: 0247713. ISSN: 0021-5120. Pub. country: Japan. Language: English.
AB Two cases of necrotizing myelopathy, one with lung carcinoma and the other with adult T cell leukemia (ATL), displayed flaccid paraplegia and sphincter dysfunction. Both cases did not show any direct neoplastic cell invasion of the spinal cord. Pathologically, diffuse and random necrosis was found in the spinal cord in both cases. In the case of ATL, small numbers of **inclusion bodies** in the nerve cell nuclei were demonstrated. Using polyclonal and also monoclonal antibodies, herpes simplex virus type 2 (HSV-2) was clearly demonstrated in the spinal cord in both cases. By electron microscopy, numerous herpes virus particles were observed in the spinal cord in both cases. The usefulness of electrophysiological studies should be emphasized in the differentiation of these cases from others which might also display flaccid paraplegia. The possibility of necrotizing myelopathy caused by HSV-2 should always be considered in the differential diagnosis of spinal cord diseases displaying flaccid paraplegia.

L45 ANSWER 12 OF 18 MEDLINE on STN
91045475. PubMed ID: 2172948. Chlamydia trachomatis pneumonitis: a case study and literature **review**. Griffin M; Pushpanathan C; Andrews W. (Dr. Charles A. Janeway Child Health Centre, Department of Pathology, Memorial University of Newfoundland, St. John's, Canada.) Pediatric pathology / affiliated with the International Paediatric Pathology Association, **(1990)** Vol. 10, No. 5, pp. 843-52. Ref: 21. Journal code: 8303527. ISSN: 0277-0938. Pub. country: United States. Language: English.
AB Chlamydia trachomatis was cultured from the lung and eye of an 11-day-old infant who had rapidly deteriorating respiratory symptoms. Chlamydia trachomatis **inclusion bodies** were identified on thick Epon sections and by electron microscopy. We discuss the unusual presentation and **review** the published histopathological lung findings.

L45 ANSWER 13 OF 18 MEDLINE on STN
86214680. PubMed ID: 3010918. Conjunctival cytology of adult chlamydial conjunctivitis. Wilhelmus K R; Robinson N M; Tredici L L; Jones D B. Archives of ophthalmology, **(1986 May)** Vol. 104, No. 5, pp. 691-3. Journal code: 7706534. ISSN: 0003-9950. Pub. country: United States. Language: English.
AB We assessed the diagnostic value of ocular cytologic examination by **reviewing** Giemsa-stained smears of conjunctival scrapings. Of 387 patients with a clinical diagnosis of adult chlamydial conjunctivitis, intracytoplasmic inclusions were found in 30 (8%). Both polymorphonuclear leukocytes and lymphocytes were common; the predominant cell type was not useful to differentiate chlamydial from adenoviral conjunctivitis. More sensitive cytologic features included the presence of plasma cells, Leber cells, blastoid cells, and multinucleated cells. Giemsa-stained conjunctival cytologic examination can provide a useful method to support the clinical diagnosis and to direct further laboratory testing.

L45 ANSWER 14 OF 18 MEDLINE on STN
81096495. PubMed ID: 553961. Parvovirus as a cause of enteritis and myocarditis in puppies. van Rensburg I B; Botha W S; Lange A L; Williams M C. Journal of the South African Veterinary Association, **(1979 Dec)** Vol. 50, No. 4, pp. 249-53. Journal code: 7503122. ISSN: 1019-9128. Pub. country: South Africa. Language: English.
AB A gastro-enteritis syndrome mimicking feline panleukopaenia was diagnosed in young dogs in the Republic of South Africa. Parvovirus was demonstrated by electron microscopy in the faeces of these animals. In addition an acutely fatal, acute to sub-acute non-purulent interstitial myocarditis occurred in pups in the same area. Histopathologically large basophilic intranuclear **inclusion bodies** were seen in the myocardium of these cases. The two syndromes generally occurred separately, but in two cases were found in the same individuals. The literature on the subject is briefly **reviewed** and the clinical and pathological findings in these outbreaks are reported.

L45 ANSWER 15 OF 18 MEDLINE on STN
79111274. PubMed ID: 105094. Possibility of a viral etiology in recurrent aphthous ulcers and Behcet's syndrome. Hooks J J. Journal of oral pathology, **(1978)** Vol. 7, No. 6, pp. 353-64. Journal code: 0342050. ISSN: 0300-9777. Pub. country: Denmark. Language: English.
AB The clinical signs, laboratory data, and histological features of recurrent aphthous ulcers (RAU) and Behcet's syndrome suggest a viral etiology. In fact, there are reports of adenovirus isolations in herpetiform oral ulcers and on the isolation of a filterable agent in sporadic cases of Behcet's syndrome. However, isolation studies on the major and minor aphthous ulcers and more recent studies on Behcet's syndrome have been negative. A **review** of the literature on the role of viruses and autoimmunity in RAU and Behcet's syndrome is presented. Biopsy specimens of ulcerative lesions were grown in vitro for up to 300 days. Those cultures, along with leukocytes and body fluids, were examined by a variety of techniques for the presence of virus or viral antigens. Although a persistent or latent virus was not detected, these negative studies cannot exclude a viral etiology. In fact, the hypothesis of an infectious and viral etiology is still reasonable.

L45 ANSWER 16 OF 18 MEDLINE on STN
78076077. PubMed ID: 595349. [Virus diseases of the bees (**review** of the literature)]. Virozy pchel.(obzor literatury). Batuev Iu M. Veterinariia, **(1977 Sep)** No. 9, pp. 108-11. Journal code: 0412751. ISSN: 0042-4846. Pub. country: USSR. Language: Russian.

L45 ANSWER 17 OF 18 MEDLINE on STN
73088744. PubMed ID: 4346432. **Review** of canine viral disease. Love D N. Australian veterinary journal, **(1972 Oct)** Vol. 48, No. 10, pp. 567-70. Ref: 47. Journal code: 0370616. ISSN: 0005-0423. Pub. country: Australia. Language: English.

L45 ANSWER 18 OF 18 MEDLINE on STN
72234168. PubMed ID: 4625472. Subacute sclerosing panencephalitis: a **review**. ter Meulen V; Katz M; Muller D. Current topics in microbiology and immunology, **(1972)** Vol. 57, pp. 1-38. Ref: 107. Journal code: 0110513. ISSN: 0070-217X. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: English.

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L5	2	S	L4 NOT L1
L6	13	S	L3 NOT L4
L7	13	S	L6 AND AY<2003
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                E SCHANTZ C/IN
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                E SEEBER S/AU
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L27           0 S L26 AND (INCLUSION BODIES)

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L29          98 S L28 AND AY<2002
L30           2 S L29 AND (MULTIPLE COP?/CLM OR MULTIPLE JOINED GENES/CLM)
L31          13 S (T1357 OR T-1357)
L32           9 S L31 AND AY<2003
L33           0 S L32 AND (T-1357/CLM OR T1357/CLM)
L34         458 S T-1249
                E BOLOGNESI D P/IN
L35          12 S E4
L36           0 S L35 AND L34
                E LAMBERT D M/IN
                E LAMBERT DENNIS M/IN
L37          22 S E3 OR E4
L38           1 S L37 AND L34
                E BARNEY S/IN
L39          22 S E7-E9
L40           1 S L39 AND L34

    FILE 'MEDLINE' ENTERED AT 12:05:13 ON 08 JAN 2007
L41       17324 S (INCLUSION BODIES)
L42        2797 S L41 AND PURIFICATION
L43        2197 S L42 AND PY<2002
L44           2 S L43 AND (MULTICOPY OR MULTIPLE COPIES OR MULTIPLE JOINED)
L45          18 S L43 AND REVIEW?

=> s l43 and (inclusion bodies/ab)
         51068 INCLUSION/AB
         61213 BODIES/AB
          4596 INCLUSION BODIES/AB
                 ((INCLUSION(W)BODIES)/AB)
L46         969 L43 AND (INCLUSION BODIES/AB)

=> s l46 and review?
        677887 REVIEW?
L47           9 L46 AND REVIEW?

=> d l47,cbib,ab,1-9

L47  ANSWER 1 OF 9     MEDLINE on STN
97422857.    PubMed ID: 9276922.   Molecular chaperones, folding catalysts, and
     the recovery of active recombinant proteins from E. coli. To fold or to
     refold. Thomas J G; Ayling A; Baneyx F. (University of Washington,
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Department of Chemical Engineering, Seattle 98195-1750, USA.) Applied biochemistry and biotechnology, **(1997 Jun)** Vol. 66, No. 3, pp. 197-238. Ref: 214. Journal code: 8208561. ISSN: 0273-2289. Pub. country: United States. Language: English.

AB The high-level expression of recombinant gene products in the gram-negative bacterium Escherichia coli often results in the misfolding of the protein of interest and its subsequent degradation by cellular proteases or its deposition into biologically inactive aggregates known as **inclusion bodies**. It has recently become clear that in vivo protein folding is an energy-dependent process mediated by two classes of folding modulators. Molecular chaperones, such as the DnaK-DnaJ-GrpE and GroEL-GroES systems, suppress off-pathway aggregation reactions and facilitate proper folding through ATP-coordinated cycles of binding and release of folding intermediates. On the other hand, folding catalysts (foldases) accelerate rate-limiting steps along the protein folding pathway such as the cis/trans isomerization of peptidyl-prolyl bonds and the formation and reshuffling of disulfide bridges. Manipulating the cytoplasmic folding environment by increasing the intracellular concentration of all or specific folding modulators, or by inactivating genes encoding these proteins, holds great promise in facilitating the production and **purification** of heterologous proteins. Purified folding modulators and artificial systems that mimic their mode of action have also proven useful in improving the in vitro refolding yields of chemically denatured polypeptides. This **review** examines the usefulness and limitations of molecular chaperones and folding catalysts in both in vivo and in vitro folding processes.

L47 ANSWER 2 OF 9 MEDLINE on STN

97149198. PubMed ID: 8996006. Cytomegalovirus as a primary pulmonary pathogen in AIDS. Waxman A B; Goldie S J; Brett-Smith H; Matthay R A. (Department of Internal Medicine, Yale University School of Medicine, New Haven, Conn 06520-8057, USA.) Chest, **(1997 Jan)** Vol. 111, No. 1, pp. 128-34. Journal code: 0231335. ISSN: 0012-3692. Pub. country: United States. Language: English.

AB In patients with AIDS, isolation of cytomegalovirus (CMV) from respiratory secretions is common. It is often found with other pathogens, which has led to debate regarding its role as a primary pulmonary pathogen. A retrospective investigation of patients with AIDS and CMV as a sole pulmonary isolate was performed in an attempt to describe their clinical presentation and course. All patients admitted to the hospital with pneumonia and with BAL or transbronchial biopsy (TBB) specimen positive for CMV between 1991 and 1994 were identified through a **review** of inpatient records. Inclusion criteria included positive CMV cultures from BAL, cytomegalic **inclusion bodies** from BAL or TBB, and thorough documentation of the absence of other pulmonary pathogens. Nine patients met the inclusion criteria for CMV pneumonitis. Seven were male and two were female, ages 26 to 44 years, and all had a history of opportunistic infections. Typical clinical presentation was characterized by increased respiratory rate, hypoxemia, and diffuse interstitial infiltrates. The mean CD4 count was 29.6 (+/- 22) cells per cubic millimeter, mean lactate dehydrogenase level was 414 (+/- 301) IU/L, and in seven patients in whom CMV antigen was measured it was greater than 50 positive cells per 200,000 WBCs. Three untreated patients died of respiratory failure and three had autopsy confirmation of CMV pneumonia. Five patients were treated with anti-CMV therapy for at least 2 weeks, and all demonstrated improvement in symptoms, oxygen saturation, and chest radiograph. At 3 months follow-up, all five patients were asymptomatic with no pulmonary symptoms. At 6 months follow-up, three of the five patients remained asymptomatic; the other two died of other opportunistic infections. In at least these nine patients, CMV represented a primary pulmonary pathogen. Patients who were treated responded quickly and were able to be discharged home from the hospital with marked improvement in their symptoms. We recommend that clinicians consider this diagnosis in the proper setting and consider treatment with anti-CMV therapy.

L47 ANSWER 3 OF 9 MEDLINE on STN

97093497. PubMed ID: 8939059. **Inclusion bodies** and **purification** of proteins in biologically active forms. Mukhopadhyay A. (National Institute of Immunology, Aruna Asaf Ali Marg, New Delhi, India.) Advances in biochemical engineering/biotechnology, **(1997)** Vol. 56, pp. 61-109. Ref: 160. Journal code: 8307733. ISSN: 0724-6145. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Even though recombinant DNA technology has made possible the production of valuable therapeutic proteins, its accumulation in the host cell as inclusion body poses serious problems in the recovery of functionally

active proteins. In the last twenty years, alternative techniques have been evolved to purify biologically active proteins from **inclusion bodies**. Most of these remain only as inventions and very few are commercially exploited. This **review** summarizes the developments in isolation, refolding and **purification** of proteins from **inclusion bodies** that could be used for vaccine and non-vaccine applications. The second section involves a discussion on **inclusion bodies**, how they are formed, and their physicochemical properties. In vivo protein folding in Escherichia coli and kinetics of in vitro protein folding are the subjects of the third and fourth sections respectively. The next section covers the recovery of bioactive protein from **inclusion bodies**: it includes isolation of inclusion body from host cell debris, **purification** in denatured state alternate refolding techniques, and final **purification** of active molecules. Since purity and safety are two important issues in therapeutic grade proteins, the following three sections are devoted to immunological and biological characterization of biomolecules, nature, and type of impurities normally encountered, and their detection. Lastly, two case studies are discussed to demonstrate the sequence of process steps involved.

L47 ANSWER 4 OF 9 MEDLINE on STN

97042090. PubMed ID: 8887361. Protein folding in vivo and renaturation of recombinant proteins from **inclusion bodies**. Guise A D; West S M; Chaudhuri J B. (School of Chemical Engineering, University of Bath, UK.) Molecular biotechnology, **(1996 Aug)** Vol. 6, No. 1, pp. 53-64. Ref: 67. Journal code: 9423533. ISSN: 1073-6085. Pub. country: United States. Language: English.

AB Eukaryotic proteins expressed in Escherichia coli often accumulate within the cell as insoluble protein aggregates or **inclusion bodies**. The recovery of structure and activity from **inclusion bodies** is a complex process, there are no general rules for efficient renaturation. Research into understanding how proteins fold in vivo is giving rise to potentially new refolding methods, for example, using molecular chaperones. In this article we **review** what is understood about the main three classes of chaperone: the Stress 60, Stress 70, and Stress 90 proteins. We also give an overview of current process strategies for renaturing **inclusion bodies**, and report the use of novel developments that have enhanced refolding yields.

L47 ANSWER 5 OF 9 MEDLINE on STN

96075435. PubMed ID: 7493381. Targeting c-erbB-2 expressing tumors using single-chain Fv monomers and dimers. Tai M S; McCartney J E; Adams G P; Jin D; Hudziak R M; Oppermann H; Laminet A A; Bookman M A; Wolf E J; Liu S; +. (Creative BioMolecules, Inc., Hopkinton, Massachusetts 01748, USA.) Cancer research, **(1995 Dec 1)** Vol. 55, No. 23 Suppl, pp. 5983s-5989s. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.

AB Single-chain Fv proteins containing a COOH-terminal cysteine (sFv') were constructed by using an antidigoxin 26.10 sFv and an anti-c-erbB-2 741F8 sFv. The fully active sFv' proteins were prepared by expression in Escherichia coli as insoluble **inclusion bodies**, followed by in vitro refolding using glutathione redox buffers and **purification**. The COOH-terminal cysteines of the refolded sFv' proteins were protected by a blocking group presumed to be the glutathionyl peptide, which was easily and selectively removed by gentle reduction. Air oxidation of the reduced sFv' monomers resulted in the efficient formation of disulfide-linked sFv' homodimers, designated (sFv')2, which were stable under oxidizing conditions and relatively slow to be disrupted under reducing conditions. The (26-10-1 sFv')-(741F8-1 sFv') heterodimer was prepared and possessed dual-antigen specificity; the active bispecific (sFv')2 dimerized under native conditions, apparently as a manifestation of self-association by the 741F8 sFv' subunit. Biodistribution and imaging studies that were performed on mice bearing human SK-OV-3 tumor xenografts that express the c-erbB-2 as a cell surface antigen were **reviewed**. Radioiodinated 741F8-2 (sFv')2 homodimer localized to the tumors with high specificity, as evidenced by excellent tumor:normal tissue ratios. Sagittal section autoradiography of whole animals 24 h after administration of antibody species revealed that 741F8 (sFv')2 produced a stronger tumor image than comparable doses of the 741F8 Fab, monomeric sFv', and the 26-10 (sFv')2 control without the high nonspecific background distribution of the 741F8 IgG.

L47 ANSWER 6 OF 9 MEDLINE on STN

93235201. PubMed ID: 8386399. [Equine granulocytic ehrlichiosis (EGE), a **review**]. Equine granulocytaire ehrlichiosis (EGE), een overzicht. van

der Kolk J H; van der Wijden M W; Jongejan F. (Vakgroep Inwendige Zieketen en Voeding der Grote Huisdieren, Faculteit Diergeneeskunde, Utrecht.) Tijdschrift voor diergeneeskunde, **(1993 Apr 1)** Vol. 118, No. 7, pp. 227-9. Ref: 30. Journal code: 0031550. ISSN: 0040-7453. Pub. country: Netherlands. Language: Dutch.

AB Equine granulocytic ehrlichiosis (EGE) has been observed in the U.S.A., Brazil, Germany, Sweden, Switzerland and possibly in Great Britain. The causative agent is rickettsia Ehrlichia equi, identified for the first time in 1969. The clinical features of the disease are anorexia, fever, depression, (limb) oedema, icterus, ataxia, petechiae and orchitis. Hematologic changes are leukopenia, thrombocytopenia, anemia and cytoplasmic **inclusion bodies** in the neutrophils and eosinophils. Vasculitis may be observed at autopsy. Following a positive hematological diagnosis (Giemsa stained blood smear) of EGE, treatment with oxytetracycline can be initiated.

L47 ANSWER 7 OF 9 MEDLINE on STN

91325466. PubMed ID: 1650859. Necrotizing myelopathy associated with malignancy caused by herpes simplex virus type 2: clinical report of two cases and literature **review**. Nakagawa M; Nakamura A; Kubota R; Kakazu T; Kuba M; Nakasone K; Iwamasa T. (Department of Neurology, National Okinawa Hospital, Ginowan, Japan.) Japanese journal of medicine, **(1991 Mar-Apr)** Vol. 30, No. 2, pp. 182-8. Journal code: 0247713. ISSN: 0021-5120. Pub. country: Japan. Language: English.

AB Two cases of necrotizing myelopathy, one with lung carcinoma and the other with adult T cell leukemia (ATL), displayed flaccid paraplegia and sphincter dysfunction. Both cases did not show any direct neoplastic cell invasion of the spinal cord. Pathologically, diffuse and random necrosis was found in the spinal cord in both cases. In the case of ATL, small numbers of **inclusion bodies** in the nerve cell nuclei were demonstrated. Using polyclonal and also monoclonal antibodies, herpes simplex virus type 2 (HSV-2) was clearly demonstrated in the spinal cord in both cases. By electron microscopy, numerous herpes virus particles were observed in the spinal cord in both cases. The usefulness of electrophysiological studies should be emphasized in the differentiation of these cases from others which might also display flaccid paraplegia. The possibility of necrotizing myelopathy caused by HSV-2 should always be considered in the differential diagnosis of spinal cord diseases displaying flaccid paraplegia.

L47 ANSWER 8 OF 9 MEDLINE on STN

91045475. PubMed ID: 2172948. Chlamydia trachomatis pneumonitis: a case study and literature **review**. Griffin M; Pushpanathan C; Andrews W. (Dr. Charles A. Janeway Child Health Centre, Department of Pathology, Memorial University of Newfoundland, St. John's, Canada.) Pediatric pathology / affiliated with the International Paediatric Pathology Association, **(1990)** Vol. 10, No. 5, pp. 843-52. Ref: 21. Journal code: 8303527. ISSN: 0277-0938. Pub. country: United States. Language: English.

AB Chlamydia trachomatis was cultured from the lung and eye of an 11-day-old infant who had rapidly deteriorating respiratory symptoms. Chlamydia trachomatis **inclusion bodies** were identified on thick Epon sections and by electron microscopy. We discuss the unusual presentation and **review** the published histopathological lung findings.

L47 ANSWER 9 OF 9 MEDLINE on STN

81096495. PubMed ID: 553961. Parvovirus as a cause of enteritis and myocarditis in puppies. van Rensburg I B; Botha W S; Lange A L; Williams M C. Journal of the South African Veterinary Association, **(1979 Dec)** Vol. 50, No. 4, pp. 249-53. Journal code: 7503122. ISSN: 1019-9128. Pub. country: South Africa. Language: English.

AB A gastro-enteritis syndrome mimicking feline panleukopaenia was diagnosed in young dogs in the Republic of South Africa. Parvovirus was demonstrated by electron microscopy in the faeces of these animals. In addition an acutely fatal, acute to sub-acute non-purulent interstitial myocarditis occurred in pups in the same area. Histopathologically large basophilic intranuclear **inclusion bodies** were seen in the myocardium of these cases. The two syndromes generally occurred separately, but in two cases were found in the same individuals. The literature on the subject is briefly **reviewed** and the clinical and pathological findings in these outbreaks are reported.

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(FILE 'HOME' ENTERED AT 11:43:32 ON 08 JAN 2007)

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                 E KOPETZKI ERHARD/IN
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                 E KOPETZKI E/IN
L14           30 S E3
L15            9 S L14 AND (INCLUSION BODIES)
L16            8 S L15 NOT L12
                 E SCHANTZ C/IN
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L18           19 S E3
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                 E KOPETZKI E/AU
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L27            0 S L26 AND (INCLUSION BODIES)

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L34          458 S T-1249
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L35           12 S E4
L36            0 S L35 AND L34
                 E LAMBERT D M/IN
                 E LAMBERT DENNIS M/IN
L37           22 S E3 OR E4
L38            1 S L37 AND L34
                 E BARNEY S/IN
L39           22 S E7-E9
L40            1 S L39 AND L34

     FILE 'MEDLINE' ENTERED AT 12:05:13 ON 08 JAN 2007
L41        17324 S (INCLUSION BODIES)
L42         2797 S L41 AND PURIFICATION
L43         2197 S L42 AND PY<2002
L44            2 S L43 AND (MULTICOPY OR MULTIPLE COPIES OR MULTIPLE JOINED)
L45           18 S L43 AND REVIEW?
L46          969 S L43 AND (INCLUSION BODIES/AB)
L47            9 S L46 AND REVIEW?

=> s l46 and (HIV or human immunodeficiency virus)
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166227 HIV
1443662 HUMAN
126045 IMMUNODEFICIENCY
424555 VIRUS
50103 HUMAN IMMUNODEFICIENCY VIRUS
(HUMAN(W)IMMUNODEFICIENCY(W)VIRUS)
L48 26 L46 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)

=> d l48,cbib,ab,1-26

L48 ANSWER 1 OF 26 MEDLINE on STN
2001472072. PubMed ID: 11516760. Recombinant fusion proteins for haemagglutination-based rapid detection of antibodies to **HIV** in whole blood. Gupta A; Gupta S; Chaudhary V K. (Department of Biochemistry, University of Delhi South Campus, Benito Juarez Road, New Delhi-110 021, India.) Journal of immunological methods, **(2001 Oct 1)** Vol. 256, No. 1-2, pp. 121-40. Journal code: 1305440. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.
AB Recombinant fusion proteins, consisting of a monovalent anti-human RBC monoclonal antibody B6, and conserved immunodominant peptide of **HIV**-1 envelope glycoprotein gp41 or **HIV**-2 envelope glycoprotein gp36, have been designed and purified after over-expression in E. coli. These fusion proteins are Fab-based and were obtained by assembling the light chain with Fd (variable domain and the first constant domain of the heavy chain) or Fd fusions containing **HIV**-derived peptide, and following a protocol of in vitro denaturation of **inclusion bodies** and subsequent renaturation to assemble functional Fab. Using a multistep column chromatographic procedure, monomeric Fab and Fab fusion proteins containing **HIV**-derived peptide were purified to high degree, free of aggregates. The yield of various proteins on the laboratory scale (1-2 l of shake flask culture) was in the range of tens of milligram. Purified anti-human RBC Fab fusion proteins containing sequences derived from **HIV**-1 gp41 and **HIV**-2 gp36 were highly specific for detection of antibodies to **HIV**-1 and **HIV**-2, respectively. The described design, expression and **purification** protocols will make it possible to produce specific recombinant reagents in large quantities for agglutination-based rapid detection of antibodies to **HIV** in whole blood.

L48 ANSWER 2 OF 26 MEDLINE on STN
2001240611. PubMed ID: 11344603. Electron microscopy of buffalo green monkey kidney cells persistently infected with hepatitis A virus and immunolocalization of HAV antigens. Basu A; Gangodkar S V; Joshi M S; Chitambar S D. (National Institute of Virology, 20A, Dr. Ambedkar Road, Pune.) Indian journal of pathology & microbiology, **(2000 Oct)** Vol. 43, No. 4, pp. 409-15. Journal code: 7605904. ISSN: 0377-4929. Pub. country: India. Language: English.
AB Studies were carried out to analyse the ultrastructural changes and the distribution of hepatitis A virus (HAV)/antigens at subcellular level in buffalo green monkey kidney (BGMK) cells persistently infected with HM-175 strain of HAV. HAV infected BGMK cells showed distinct abnormalities in the endoplasmic reticulum and cytoplasmic membrane as compared to uninfected cells. The abnormalities were characterized by wavy arrays, structures like myelin, annulate lamellae, cytoplasmic **inclusion bodies** and vesicles. The wavy arrays within the cytoplasm of the host cells appeared to represent degenerating membranes. A complex myelin like body was found in close association with a group of virus like particles. Annulate lamellae like structures involving single paired membrane were detected infrequently whereas the cytoplasmic vesicles were numerous in these cells. An indirect immunogold technique was utilized to localize the HAV antigenin infected cells. A high density immunogold label for **HIV** like particles was predominantly detected in cytoplasmic vesicles. These results suggest a strong association of membrane substructure in vesicle forms with the compartmentalized replication of HAV within persistently infected host cells.

L48 ANSWER 3 OF 26 MEDLINE on STN
2000239517. PubMed ID: 10793163. Testing for polyomavirus type BK DNA in plasma to identify renal-allograft recipients with viral nephropathy. Nickeleit V; Klimkait T; Binet I F; Dalquen P; Del Zenero V; Thiel G; Mihatsch M J; Hirsch H H. (Institute for Pathology, University of Basel, Switzerland.) The New England journal of medicine, **(2000 May 4)** Vol. 342, No. 18, pp. 1309-15. Journal code: 0255562. ISSN: 0028-4793. Pub. country: United States. Language: English.
AB BACKGROUND: Reactivation of polyomavirus type BK (BK virus) is increasingly recognized as a cause of severe renal-allograft dysfunction.

Currently, patients at risk for nephropathy due to infection with the BK virus are identified by the presence of cells containing viral **inclusion bodies** ("decoy cells") in the urine or by biopsy of allograft tissue. METHODS: In a retrospective analysis, we performed polymerase-chain-reaction assays for BK virus DNA in plasma samples from 9 renal-allograft recipients with BK virus nephropathy; 41 renal-allograft recipients who did not have signs of nephropathy, 16 of whom had decoy cells in the urine; and as immunocompromised controls, 17 patients who had **human immunodeficiency virus** type 1 (**HIV**-1) infection (stage C3 according to the classification of the Centers for Disease Control and Prevention) and who had not undergone transplantation. RESULTS: In all nine patients with BK virus nephropathy, BK virus DNA was detected in the plasma at the time of the initial histologic diagnosis (a mean [+/-SD] of 46+/-28 weeks after transplantation) and during the course of histologically diagnosed, persistent BK virus disease. In three of the six patients with nephropathy who were studied serially after transplantation, BK virus DNA was initially undetectable but was detected 16 to 33 weeks before nephropathy became clinically evident and was confirmed by biopsy. Tests for BK virus DNA in plasma became negative and the nephropathy resolved after the doses of immunosuppressive drugs were decreased in two patients and after removal of the renal allograft in three patients. BK virus DNA was found in the plasma of only 2 of the 41 renal-allograft recipients who had no signs of nephropathy and in none of the patients with **HIV**-1 infection. CONCLUSIONS: Testing for BK virus DNA in plasma from renal-allograft recipients with use of the polymerase chain reaction is a sensitive and specific method for identifying viral nephropathy.

L48 ANSWER 4 OF 26 MEDLINE on STN

1999350756. PubMed ID: 10419833. High-level expression and **purification** of biologically active recombinant pokeweed antiviral protein. Rajamohan F; Engstrom C R; Denton T J; Engen L A; Kourinov I; Uckun F M. (Biotherapy Program, Hughes Institute, St. Paul, Minnesota 55113, USA.) Protein expression and purification, **(1999 Jul)** Vol. 16, No. 2, pp. 359-68. Journal code: 9101496. ISSN: 1046-5928. Pub. country: United States. Language: English.

AB Pokeweed antiviral protein (PAP) from the leaves of the pokeweed plant, Phytolacca americana, is a naturally occurring single-chain ribosome-inactivating protein, which catalytically inactivates both prokaryotic and eukaryotic ribosomes. The therapeutic potential of PAP has gained considerable interest in recent years due to the clinical use of native PAP as the active moiety of immunoconjugates against cancer and AIDS. The clinical use of native PAP is limited due to inherent difficulties in obtaining sufficient quantities of a homogenously pure and active PAP preparation with minimal batch to batch variability from its natural source. Previous methods for expression of recombinant PAP in yeast, transgenic plants and Escherichia coli have resulted in either unacceptably low yields or were too toxic to the host system. Here, we report a successful strategy which allows high level expression of PAP as **inclusion bodies** in E. coli. **Purification** of refolded recombinant protein from solubilized **inclusion bodies** by size-exclusion chromatography yielded biologically active recombinant PAP (final yield: 10 to 12 mg/L). The ribosome depurinating in vitro N-glycosidase activity and cellular anti-**HIV** activity of recombinant PAP were comparable to those of the native PAP. This expression and **purification** system makes it possible to obtain sufficient quantities of biologically active and homogenous recombinant PAP sufficient to carry out advanced clinical trials. To our knowledge, this is the first large-scale expression and **purification** of biologically active recombinant PAP from E. coli.

L48 ANSWER 5 OF 26 MEDLINE on STN

97322324. PubMed ID: 9177169. Assembly of a rod-shaped chimera of a trimeric GCN4 zipper and the **HIV**-1 gp41 ectodomain expressed in Escherichia coli. Weissenhorn W; Calder L J; Dessen A; Laue T; Skehel J J; Wiley D C. (Laboratory of Molecular Medicine, The Children's Hospital, 320 Longwood Avenue, Boston, MA 02215, USA.) Proceedings of the National Academy of Sciences of the United States of America, **(1997 Jun 10)** Vol. 94, No. 12, pp. 6065-9. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB The **HIV**-1 envelope subunit gp41 plays a role in viral entry by initiating fusion of the viral and cellular membranes. A chimeric molecule was constructed centered on the ectodomain of gp41 without the fusion peptide, with a trimeric isoleucine zipper derived from GCN4 (pIIGCN4) on the N terminus and part of the trimeric coiled coil of the influenza virus hemagglutinin (HA) HA2 on the C terminus. The chimera

pII-41-HA was overexpressed as **inclusion bodies** in bacteria and refolded to soluble aggregates that became monodisperse after treatment with protease. Either trypsin or proteinase K, used previously to define a protease-resistant core of recombinant gp41 [Lu, M., Blacklow, S. C. & Kim, P. S. (1995) Nat. Struct. Biol. 2, 1075-1082], removed about 20-30 residues from the center of gp41 and all or most of the HA2 segment. Evidence is presented that the resulting soluble chimera, retaining the pIIGCN4 coiled coil at the N terminus, is an oligomeric highly alpha-helical rod about 130 A long that crystallizes. The chimeric molecule is recognized by the Fab fragments of mAbs specific for folded gp41. A similar chimera was assembled from the two halves of the molecule expressed separately in different bacteria and refolded together. Crystals from the smallest chimera diffract x-rays to 2.6-A resolution.

L48 ANSWER 6 OF 26 MEDLINE on STN

97254966. PubMed ID: 9100348. **HIV**-I protease. Cloning, expression, and **purification**. Dergousova N I; Amerik AYu; Volynskaya A M; Rumsh L D. (Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia.. nd@enzyme.siobc.ras.ru) . Applied biochemistry and biotechnology, **(1996 Oct-Nov)** Vol. 61, No. 1-2, pp. 97-107. Journal code: 8208561. ISSN: 0273-2289. Pub. country: United States. Language: English.

AB A new method for obtaining **HIV**-I protease was suggested. Fusion proteins composed of the N-terminal fragment of human gamma-interferon and **HIV**-I protease connected with (Asp)4Lys (protein I) or Asp-Pro (protein II) linkers were expressed in Escherichia coli cells. The fusion proteins were produced as insoluble **inclusion bodies** in the 20% yield of total cell protein. Protein I was cleaved by enterokinase. The solubility of protein I was increased by treating with Na-sulfite/Na-tetrathionate under denaturing conditions. Optimal conditions for efficient acidic hydrolysis of protein II at Asp-Pro bond were found. The hydrolysis products were separated by reversed-phase FPLC. The amount of tryptophan and cysteine residues in the enzyme obtained was estimated. The activity of **HIV**-I protease was determined using the chromogenic peptide. AlaArgVal NleNphGluAlaNleNH2 and a high-mol-wt substrate consisting of beta-galactosidase and a fragment of gag proteins, including p17-p24 processing site.

L48 ANSWER 7 OF 26 MEDLINE on STN

97046153. PubMed ID: 8891075. Rabies encephalitis in a patient with AIDS: a clinicopathological study. Adle-Biassette H; Bourhy H; Gisselbrecht M; Chretien F; Wingertsmann L; Baudrimont M; Rotivel Y; Godeau B; Gray F. (Departement de Pathologie (Neuropathologie), Hopital Henri Mondor, Creteil, France.) Acta neuropathologica, **(1996 Oct)** Vol. 92, No. 4, pp. 415-20. Journal code: 0412041. ISSN: 0001-6322. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB A 46-year-old man was bitten by a dog in Mali; anti-rabies vaccination was incomplete. Three months later he was admitted to hospital with fever and diarrhea. **Human immunodeficiency virus** (**HIV**) serology was positive and CD4 count was 70/mm3. His status worsened rapidly with confusion hydrophobia and hypersialorrhea. Despite anti-rabies serotherapy and vaccination, he died suddenly 12 days after admission. Immunofluorescence on cerebral tissue samples established rabies encephalitis. Neuropathology showed mild encephalitis with occasional Babes nodules and rare perivascular mononuclear cuffs. Intraneuronal Negri **inclusion bodies** were remarkably diffuse and abundant. They were clearly demonstrated by immunocytochemistry and electron microscopy. Apoptotic neurons were identified in the brain stem and hippocampus in the vicinity of inflammatory foci. In contrast, apoptosis could not be demonstrated in non-inflammatory areas, even where Negri bodies were numerous. There was no associated **HIV** encephalitis or opportunistic infection. The occurrence of rabies encephalitis in AIDS represents a random association, but is probably not exceptional as rabies is endemic in many countries and the AIDS epidemic is spreading worldwide. In this case, although the incubation duration and clinical presentation were comparable to those in classical rabies the T-cell-mediated immunosuppression may account for the weak inflammatory reaction and unusually abundant viral multiplication. This observation confirms that all those at risk for rabies, particularly immunocompromised patients, should receive complete anti-rabies treatment including vaccines and specific immunoglobulins, as soon as possible after infection.

L48 ANSWER 8 OF 26 MEDLINE on STN

96136639. PubMed ID: 8527938. Large-scale production of **HIV**-1 protease from Escherichia coli using selective extraction and membrane

fractionation. Gustafson M E; Junger K D; Foy B A; Baez J A; Bishop B F; Rangwala S H; Michener M L; Leimgruber R M; Houseman K A; Mueller R A; +. (G.D. Searle and Co., Chesterfield, Missouri 63198, USA.) Protein expression and purification, **(1995 Aug)** Vol. 6, No. 4, pp. 512-8. Journal code: 9101496. ISSN: 1046-5928. Pub. country: United States. Language: English.

AB **Human immunodeficiency virus** type 1 (**HIV**-1) protease was expressed in Escherichia coli as a fusion protein with the N-terminal sequence of IGF-2. The protein accumulated in **inclusion bodies** as a 40:60 mixture of unprocessed fusion protein and processed protein. A simple **purification** procedure was developed that yielded 30-40 mg of active protease per liter of fermentation broth with a recovery of 30-40%. The **purification** process involved the selective extraction of **HIV**-1 protease from E. coli **inclusion bodies** with 50% acetic acid and fractional diafiltration to remove impurities and low-molecular-weight protease-related fragments. No chromatographic steps were employed, yet the **HIV**-1 protease produced by this procedure was greater than 95% pure by SDS-PAGE, reverse-phase HPLC, and N-terminal sequence analysis.

L48 ANSWER 9 OF 26 MEDLINE on STN

95353236. PubMed ID: 7627139. Expression and **purification** of active form of **HIV**-1 protease from E.coli. Wan M; Loh B N. (Department of Biochemistry, Faculty of Medicine, National University of Singapore, Kent Ridge Crescent.) Biochemistry and molecular biology international, **(1995 Apr)** Vol. 35, No. 4, pp. 899-912. Journal code: 9306673. ISSN: 1039-9712. Pub. country: Australia. Language: English.

AB We have subcloned an N-terminal extended protease gene of **human immunodeficiency virus** (**HIV**) type 1 that is encoded in the protease domain of the pol open reading frame into expression vector pGEX-KG. A relatively high level of expression of recombinant **HIV**-1 protease (PR) was achieved with isopropyl beta-D-thiogalactoside (IPTG) induction and glucose supplement. An isolation method consisting of denaturation of protein and followed by refolding was developed for releasing this recombinant **HIV**-1 PR into the soluble phase since most of the expressed protease was initially present in insoluble **inclusion bodies**. High purity of this recombinant **HIV**-1 PR was obtained by sequential **purification** using Sephadex G-50 gel filtration and CM-23 cellulose cation exchange chromatography, yielding the protease more than 1 mg per liter culture. N-terminal amino acid sequence analysis showed that the recombinant **HIV**-1 PR underwent autocleavage from the fusion protein during expression. SDS-PAGE indicated that the molecular weight of this recombinant **HIV**-1 PR is 11 kDa. This recombinant **HIV**-1 PR showed proteolytic activity for the synthetic peptide substrates corresponding to the sequence at the Gag MA/CA and Pol p6*/PR junctions. The purified enzyme whose specific activity for the heptapeptide SQNYPIV was 848.7 nmol*min-1*mg protease-1 also processed recombinant polyprotein Gag41 as its substrate.

L48 ANSWER 10 OF 26 MEDLINE on STN

95268877. PubMed ID: 7749785. The morphogenesis of a Chinese strain of **HIV**-1 forming **inclusion bodies** in Jurkat-tat III cells. Li Q G; Zhang Y J; Liang Y; Feng C Q; Li Y Z; Sjoberg R; Jiang Y; Wang N F; Wadell G. (Department of Virology, Institute of Infectious Diseases, Beijing, China.) Journal of acquired immune deficiency syndromes and human retrovirology : official publication of the International Retrovirology Association, **(1995 Jun 1)** Vol. 9, No. 2, pp. 103-13. Journal code: 9501482. ISSN: 1077-9450. Pub. country: United States. Language: English.

AB A rapid/high replicative strain of **human immunodeficiency virus** type 1 (**HIV**-1) (BC9101) was isolated directly in the Jurkat-tat III cell line from a Chinese patient with AIDS. The thin-section electron microscopy was performed and revealed high efficiency of replication of BC9101 with some unusual biological properties. Many vacuoles, most of them filled with **HIV** particles, were found close to the nucleus. Double-cored virions and double budding were frequently observed in the vacuoles and at the vacuolar membrane. Virus particles matured by budding both into intracytoplasmic vacuoles and through the plasma membrane. **Inclusion bodies** of varying sizes, some consisting of thousands of **HIV** particles, were found in the cytoplasm. All the illustrated features describing formation of **inclusion bodies** were compatible with the observation that **HIV** particles were assembled at and budded from the cytoplasmic vacuole membrane. They were then released from the membrane into the vacuoles, and subsequently, the maturation occurred. Some of the vacuoles accumulated to such a high number of mature virus particles that **inclusion bodies** were formed. During the disintegration of the cells, the **inclusion bodies** surrounded by the vacuolar membrane were released

from the cells. The nucleotide sequence of the vpu gene of BC9101 was investigated and indicated that the unusual biological properties may due to the lack of a start codon for translation of the vpu protein.

L48 ANSWER 11 OF 26 MEDLINE on STN
95229748. PubMed ID: 7714031. A recombinant **human immunodeficiency virus** type-1 capsid protein (rp24): its expression, **purification** and physico-chemical characterization. Hausdorf G; Gewiess A; Wray V; Porstmann T. (Institut fur Biochemie der Charite Humboldt-Universitat, Medizinische Fakultat (Charite), Med. Universit atsklinik III, Berlin, Germany.) Journal of virological methods, **(1994 Dec)** Vol. 50, No. 1-3, pp. 1-9. Journal code: 8005839. ISSN: 0166-0934. Pub. country: Netherlands. Language: English.
AB An expression system has been established in Escherichia coli to facilitate the preparation of the **HIV**-1 capsid protein in amounts sufficient for structural analysis. A plasmid vector pTCA5, containing the gene for the recombinant **HIV**-1 capsid protein rp24 under the control of the lambda-PR-promoter, was constructed which gave an expression product that spanned 234 amino acid residues. It differs at the N-terminus from the authentic sequence in that the residues Pro-Ile- are replaced by Met-Asn-Ser-Ala-Met-. Recombinant p24 was produced, as **inclusion bodies** in E. coli LE392 containing pTCA5, at a level of approximately 15% of the total cellular protein. After dissolution of the **inclusion bodies** in the acidic urea system, the protein was easily reconstituted in a soluble state by dialysis. The yield of reconstituted and purified protein was 12 mg per liter in rich medium. Recombinant rp24 consists of about 40% alpha-helix and 10% beta-sheet from circular dichroism measurements and the two cysteine residues, within the rp24 sequence, are bridged by a disulfide bond.

L48 ANSWER 12 OF 26 MEDLINE on STN
94263515. PubMed ID: 7764847. Overexpression and simple **purification** of **human immunodeficiency virus**-1 gag epitope derived from a recombinant antigen in E. coli and its use in ELISA. Sohn M J; Chong Y H; Chang J E; Lee Y I. (Molecular Genetics Lab, Korea Institute of Science and Technology, Taejon, South Korea.) Journal of biotechnology, **(1994 May 15)** Vol. 34, No. 2, pp. 149-55. Journal code: 8411927. ISSN: 0168-1656. Pub. country: Netherlands. Language: English.
AB To develop a test for diagnosis of **human immunodeficiency virus**-1 (**HIV**-1) exposure sensitivity, a part of the gag gene was cloned and expressed in Escherichia coli, using expression vectors containing a trp promoter. The immunoreactivity of recombinant protein was determined using **HIV**-1 specific antibodies in a Western blot analysis. The recombinant plasmid, pYHCgag3, gag gene was fused to the trpE' gene linked to the hydroxylamine (HA) cleavage recognition sequence which was induced to overexpress a core antigen (gag a.a. 121-398 from plasmid BH10) as fusion protein in the form of insoluble inclusion body. Recombinant gag was purified by a simple single step **purification** procedure. After partial **purification** of **inclusion bodies** and subject to the HA-cleavage treatment, gag protein was further purified to homogeneity using DEAE-Sepharose chromatography. The purified core antigen offered reliable results with high sensitivity and specificity for identification of **HIV**-1 antibodies when tested in the enzyme-linked immunosorbent assay (ELISA). These results suggest that mass production of recombinant core antigen will provide a valuable resource to **HIV**-1 serodiagnostics for the screening of large groups of blood donors to prevent **HIV**-1 infection.

L48 ANSWER 13 OF 26 MEDLINE on STN
94220860. PubMed ID: 8167477. **Purification** of crystallizable recombinant SIVmac251-32H proteinase. Sugrue R J; Almond N; Kitchin P; Richardson S M; Wilderspin A F. (Department of Crystallography, Birkbeck College, London, United Kingdom.) Protein expression and purification, **(1994 Feb)** Vol. 5, No. 1, pp. 76-83. Journal code: 9101496. ISSN: 1046-5928. Pub. country: United States. Language: English.
AB We have cloned a simian immunodeficiency virus (SIV) proteinase gene directly from proviral DNA of the infectious viral stock SIVmac251-32H (11/88 pool). The deduced amino acid sequence from this proteinase gene is similar to that for the published SIVmac239 molecular clone. SIVmac251-32H proteinase (SIV PR) and its flanking pol sequences were expressed in Escherichia coli as a fusion protein with most of the T7 bacteriophage gene 10 protein. The expressed protein formed cytoplasmic **inclusion bodies** which were solubilized in 8 M urea, and the recombinant SIV PR was refolded, yielding active, self-processed enzyme. The SIV PR was purified to homogeneity using a single pepstatin A affinity chromatography step, and had a specific peptidolytic activity of 20

mumol/min/mg. Enzymatic characteristics similar to those previously documented for other immunodeficiency virus proteinases (EC 3.4.23) were observed. These include an acidic pH optimum (pH 5.3), sensitivity to sodium chloride concentration, and complete inhibition by pepstatin A. In addition to these properties we have observed quantitative crystallization from low protein concentrations. We describe the first crystal habit for the proteinase from the **HIV**-2/SIV class of immunodeficiency virus, which is distinctly different from that for **HIV**-1 proteinase crystals.

L48 ANSWER 14 OF 26 MEDLINE on STN

94216501. PubMed ID: 8163659. Identification of a novel simian parvovirus in cynomolgus monkeys with severe anemia. A paradigm of human B19 parvovirus infection. O'Sullivan M G; Anderson D C; Fikes J D; Bain F T; Carlson C S; Green S W; Young N S; Brown K E. (Comparative Medicine Clinical Research Center, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, North Carolina 27157-1040.) The Journal of clinical investigation, **(1994 Apr)** Vol. 93, No. 4, pp. 1571-6. Journal code: 7802877. ISSN: 0021-9738. Pub. country: United States. Language: English.

AB Although human B19 parvovirus infection has been clearly associated with a number of distinct syndromes (including severe anemia, abortion, and arthritis), detailed knowledge of its pathogenesis has been hindered by the lack of a suitable animal model. We have identified a novel simian parvovirus in cynomolgus monkeys with severe anemia. Sequencing of a 723-bp fragment of cloned viral DNA extracted from serum revealed that the simian parvovirus has 65% homology at the DNA level with the human B19 parvovirus but little homology with other known parvoviruses. Light microscopic examination of bone marrow from infected animals showed intranuclear **inclusion bodies**, and ultrastructural studies showed viral arrays characteristic of parvoviruses. Another striking feature was the presence of marked dyserythropoiesis in cells of the erythroid lineage, raising the possibility that B19 parvovirus infection may underlie related dyserythropoietic syndromes in human beings. Affected animals had concurrent infection with the immunosuppressive type D simian retrovirus, analogous to **HIV** patients who develop severe anemia because of infection with B19 parvovirus. The remarkable similarities between the simian and B19 parvoviruses suggest that experimentally infected cynomolgus monkeys may serve as a useful animal model of human B19 infection.

L48 ANSWER 15 OF 26 MEDLINE on STN

94144349. PubMed ID: 8310822. Acute varicella-zoster virus ventriculitis and meningo-myelo-radiculitis in acquired immunodeficiency syndrome. Chretien F; Gray F; Lescs M C; Geny C; Dubreuil-Lemaire M L; Ricolfi F; Baudrimont M; Levy Y; Sobel A; Vinters H V. (Department de Pathologie (Neuropathologie), Hopital Henri Mondor, Faculte de Medecine de Creteil, Universite Paris XII, France.) Acta neuropathologica, **(1993)** Vol. 86, No. 6, pp. 659-65. Journal code: 0412041. ISSN: 0001-6322. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB A 30-year-old AIDS patient with no history of cutaneous eruption, presented with rapidly progressive flaccid paraplegia, hypoesthesia, urinary retention, moderate psychomotor slowing and fever (39.8 degrees C), leading to death within 1 week. CD4 count was 290/mm3. Cerebrospinal fluid contained 210 white blood cells and 238 mg/100 ml protein. Neuropathology revealed **HIV** encephalitis and diffuse ventriculitis with Cowdry type A inclusions in the ependymal cells. Extensive necrotic and hemorrhagic changes with marked recrotizing vasculitis involved the entire spinal cord and spinal roots. Immunocytochemistry revealed numerous **inclusion bodies** positive for varicella-zoster virus (VZV) and negative for cytomegalovirus (CMV) and herpes simplex virus type 1 and 2, in ependymal cells, subpial glial cells, endothelial cells and Schwann cells. Electron microscopy confirmed herpes virus-like particles. In situ hybridization confirmed VZV genome in leptomeninges, brain, spinal cord and spinal roots. Comparable neuropathological findings and numerous VZV **inclusion bodies** were also found in the brain, spinal cord, and spinal roots of a 40-year-old AIDS patient who died from a fulminant ascending myeloradiculopathy previously reported as "necrotizing vasculitis of the nervous system". Direct infection of the brain by VZV, in AIDS patients, has been shown to cause leukoencephalitis and cerebral non-inflammatory vasculopathies. Our observations demonstrate that, in AIDS patients, VZV infection of the central nervous system may also be responsible for meningo-myelo-radiculitis possibly secondary to ventriculitis as in CMV infection. The role of VZV in the pathogenesis of some AIDS-related vasculitides seems also very likely.

L48 ANSWER 16 OF 26 MEDLINE on STN

94000339. PubMed ID: 8397790. Large scale **purification** and refolding of **HIV**-1 protease from Escherichia coli **inclusion bodies**. Hui J O; Tomasselli A G; Reardon I M; Lull J M; Brunner D P; Tomich C S; Heinrikson R L. (Upjohn Company, Kalamazoo, Michigan 49001.) Journal of protein chemistry, **(1993 Jun)** Vol. 12, No. 3, pp. 323-7. Journal code: 8217321. ISSN: 0277-8033. Pub. country: United States. Language: English.

AB The protease encoded by the **human immunodeficiency virus** type 1 (**HIV**-1) was engineered in Escherichia coli as a construct in which the natural 99-residue polypeptide was preceded by an NH2-terminal methionine initiator. **Inclusion bodies** harboring the recombinant **HIV**-1 protease were dissolved in 50% acetic acid and the solution was subjected to gel filtration on a column of Sephadex G-75. The protein, eluted in the second of two peaks, migrated in SDS-PAGE as a single sharp band of M(r) approximately 10,000. The purified **HIV**-1 protease was refolded into an active enzyme by diluting a solution of the protein in 50% acetic acid with 25 volumes of buffer at pH 5.5. This method of **purification**, which has also been applied to the **purification** of **HIV**-2 protease, provides a single-step procedure to produce 100 mg quantities of fully active enzyme.

L48 ANSWER 17 OF 26 MEDLINE on STN

93314803. PubMed ID: 8325379. Cloning, expression and **purification** of a recombinant poly-histidine-linked **HIV**-1 protease. Leuthardt A; Roesel J L. (Ciba-Geigy Ltd., Oncology and Virology Research Department, Basel, Switzerland.) FEBS letters, **(1993 Jul 12)** Vol. 326, No. 1-3, pp. 275-80. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.

AB The gene coding for the **HIV**-1 protease was cloned in an Escherichia coli expression vector adding three-histidine codons to the amino and carboxy terminus of the protease sequence. Expression of the protease from this construct led to the accumulation of high amounts of insoluble histidine-linked protease entrapped in **inclusion bodies**. The histidine-linked protease could be efficiently released from purified **inclusion bodies** with 6 M guanidine hydrochloride and further purified by metal chelate affinity chromatography. The refolded protease cleaved synthetic peptide substrates and the viral polyprotein p55 with the same specificity as the wild type protease. It displays a specific activity of 4.4 mumol/min/mg.

L48 ANSWER 18 OF 26 MEDLINE on STN

92297315. PubMed ID: 1368241. High-level expression and **purification** of mature **HIV**-1 protease in Escherichia coli under control of the araBAD promoter. Taylor A; Brown D P; Kadam S; Maus M; Kohlbrenner W E; Weigl D; Turon M C; Katz L. (Corporate Molecular Biology, Abbott Laboratories, IL 60064.) Applied microbiology and biotechnology, **(1992 May)** Vol. 37, No. 2, pp. 205-10. Journal code: 8406612. ISSN: 0175-7598. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB A 1.3-kb segment of Escherichia coli DNA containing the regulatory gene, araC, and the promoter of the araBAD operon was amplified by the polymerase chain reaction (PCR) and cloned into pUC18, resulting in plasmid pKB130 that produced the alpha fragment of beta-galactosidase upon addition of L-arabinose (L-ara). A synthetic gene for **human immunodeficiency virus** (**HIV**)-1 preprotease was placed downstream of the ara-BAD promoter in pKB130 to create a translational fusion inducible by addition of L-ara. The fusion protein correctly autoprocessed in vivo to yield a mature 99-amino-acid **HIV**-1 protease, which was found predominantly in **inclusion bodies**. This material could be refolded to an active form, which was purified to homogeneity. A small fraction of the protease was expressed in vivo as a soluble active form, which allowed the monitoring of expression during fermentation by a rapid and simple whole cell assay employing an **HIV**-1 protease-specific fluorogenic substrate.

L48 ANSWER 19 OF 26 MEDLINE on STN

92241455. PubMed ID: 1315296. Cytomegalovirus (CMV) pneumonitis in AIDS patients: the result of intensive CMV replication?. Aukrust P; Farstad I N; Froland S S; Holter E. (Medical Dept A, University of Oslo, National Hospital, Norway.) The European respiratory journal : official journal of the European Society for Clinical Respiratory Physiology, **(1992 Mar)** Vol. 5, No. 3, pp. 362-4. Journal code: 8803460. ISSN: 0903-1936. Pub. country: Denmark. Language: English.

AB We report a case of fatal pulmonary disease in a patient infected with **human immunodeficiency virus** (**HIV**), where cytomegalovirus (CMV) was the only causative agent identified in the lungs at autopsy. The most

prominent histopathological features were numerous interalveolar cells containing CMV **inclusion bodies** combined with scanty signs of inflammation. We propose that the lung damage caused by CMV in acquired immune deficiency syndrome (AIDS) patients is a direct consequence of cytopathogenic effects of the virus related to the extent of active virus replication.

L48 ANSWER 20 OF 26 MEDLINE on STN

91353038. PubMed ID: 1882519. [The expression of a fragment of the **HIV**-1 env gene in the cells of Escherichia coli bacteria]. Ekspressiia fragmenta gena env VICh-1 v kletkakh bakterii Escherichia coli. Kazennova E V; Bobkova M R; Bobkov A F; Sergeev O V; Lukashevich N V; Garaev M M. Voprosy virusologii, **(1991 Mar-Apr)** Vol. 36, No. 2, pp. 122-5. Journal code: 0417337. ISSN: 0507-4088. Pub. country: USSR. Language: Russian.

AB A recombinant plasmid pEK6 determining the synthesis of a hybrid protein the N-terminus of which was represented by full-size beta-galactosidase and C-terminus by **HIV**-1 gene env virus-specific sequence was constructed. The analysis of lysates of E. coli HB101/pEK6 bacteria in 6% PAAGE revealed additional proteins with molecular weights from 185 to 130 kDa. These proteins interacted with blood serum antibodies of a virus carrier but formed no specific bands with sera from normal donors. Densitometric analysis of polyacrylamide gels stained with Coomassi R250 demonstrated that the level of production of recombinant protein was at least 15% of the total cell protein. Hybrid polypeptides formed poorly soluble **inclusion bodies** in the bacterial cells. Study of the immunological properties of the recombinant polypeptides showed that immunization of rabbits with these proteins induced antibodies specifically reacting with viral polypeptides with molecular weights of about 82 and 140 kDa. Such features as a high level of synthesis, technologically feasible **purification** of **inclusion bodies**, and adequate antigenic properties recommend this preparation for use in the development of diagnostic test systems.

L48 ANSWER 21 OF 26 MEDLINE on STN

91139757. PubMed ID: 2286639. **Purification** of a recombinantly produced transmembrane protein (gp41) of **HIV** I. Soutschek E; Hoflacher B; Motz M. (Mikrogen GmbH, Munchen 2 F.R.G.) Journal of chromatography, **(1990 Nov 23)** Vol. 521, No. 2, pp. 267-77. Journal code: 0427043. ISSN: 0021-9673. Pub. country: Netherlands. Language: English.

AB The transmembrane protein gp41, a component of the viral envelope of **HIV** I, and its analogue gp36 of **HIV** II are important antigens for the sensitive and specific detection of anti-**HIV** antibodies. The immunodominant region of the protein gp41, which reacts with 100% of sera of infected persons, was produced by gene technological means in Escherichia coli. The protein accumulates in the form of insoluble **inclusion bodies** in the bacterial cell. **Purification** strategies for this aggregated material depend mainly on the isolation of these "**inclusion bodies**" and subsequent washing procedures. Growth conditions of the recombinant E. coli cells and the method of the cell disruption are important for the efficiency of **purification** and the recovery of the antigen. Owing to the insolubility of the expressed antigen, a significant concentration of recombinant gp41 was possible by extracting the soluble cell components. For this purpose, mild detergent solutions and low-molarity chaotropic buffer solutions were used. After final solubilization in 8 M urea buffer at pH 12.5, further chromatographic **purification** steps followed. The reduction of disulphide bridges with beta-mercaptoethanol or dithiothreitol was important. Gel filtration on a Sephacryl S-200 or Superose 12 column and/or ion-exchange chromatography on a DEAE-Sepharose Fast Flow or Mono Q HR (5/5) column finally resulted in the desired purity of the antigen.

L48 ANSWER 22 OF 26 MEDLINE on STN

90236316. PubMed ID: 2158928. High-level synthesis of recombinant **HIV**-1 protease and the recovery of active enzyme from **inclusion bodies**. Cheng Y S; McGowan M H; Kettner C A; Schloss J V; Erickson-Viitanen S; Yin F H. (Central Research and Development Department, E.I. duPont de Nemours and Co., Wilmington, DE 19880-0328.) Gene, **(1990 Mar 15)** Vol. 87, No. 2, pp. 243-8. Journal code: 7706761. ISSN: 0378-1119. Pub. country: Netherlands. Language: English.

AB A complete chemical synthesis and assembly of genes for the production of **human immunodeficiency virus** type-I protease (**HIV**-PR) and its precursors are described. The T7 expression system was used to produce high levels of active **HIV**-PR and its precursors in Escherichia coli **inclusion bodies**. The gene encoding the open reading frames of **HIV**-PR was expressed in E. coli as a 10-kDa protein, while the genes

encoding **HIV**-PR precursors were expressed as larger proteins, which were partially processed in E. coli to the 10-kDa form. These processing events are autoproteolytic, since a single-base mutation, changing the active-site aspartic acid to glycine, completely abolished the conversion. **HIV**-PR can be released with 8 M urea from washed cellular **inclusion bodies**, resulting in a preparation with few bacterial host proteins. After refolding, this preparation contains no nonspecific protease or peptidase activities. The recombinant **HIV**-PR isolated from **inclusion bodies** cleaves **HIV**-PR substrates specifically with a specific activity comparable to column-purified **HIV**-PR.

L48 ANSWER 23 OF 26 MEDLINE on STN

90212579. PubMed ID: 2182116. Substrate analogue inhibition and active site titration of purified recombinant **HIV**-1 protease. Tomasselli A G; Olsen M K; Hui J O; Staples D J; Sawyer T K; Heinrikson R L; Tomich C S. (Biopolymer Chemistry, Research Unit, Upjohn Company, Kalamazoo, Michigan 49001.) Biochemistry, **(1990 Jan 9)** Vol. 29, No. 1, pp. 264-9. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB The aspartyl protease of **human immunodeficiency virus** 1 (**HIV**-1) has been expressed in Escherichia coli at high levels, resulting in the formation of **inclusion bodies** which contain denatured insoluble aggregates of the protease. After solubilization of these **inclusion bodies** in guanidinium chloride, the protease was purified to apparent homogeneity by a single-step reverse-phase HPLC procedure. The purified, but inactive, protein was denatured in 8 M urea and refolded to produce the active protease. Enzyme activity was demonstrated against the substrate H-Val-Ser-Gln-Asn-Tyr-Pro-Ile-Val-OH, modeled after the cleavage region between residues 128 and 135 in the **HIV** gag polyprotein. With this substrate, a Vmax of 1.3 +/- 0.2 mumol/(min.mg) and KM of 2.0 +/- 0.3 mM were determined at pH 5.5. Pepstatin (Iva-Val-Val-Sta-Ala-Sta-OH) and substrate analogues with the Tyr-Pro residues substituted by Sta, by Phe psi [CH2N]Pro, and by Leu psi [CH(OH)CH2]Val inhibited the protease with KI values of 360 nM, 3690 nM, 3520 nM, and less than 10 nM, respectively. All were competitive inhibitors, and the tightest binding compound provided an active site titrant for the quantitative determination of enzymatically active **HIV**-1 protease.

L48 ANSWER 24 OF 26 MEDLINE on STN

90065473. PubMed ID: 2555629. [Diagnosis and clinical aspects of gastrointestinal cytomegalovirus diseases in patients with **human immunodeficiency virus** 1 infection]. Diagnostik und Klinik gastrointestinaler Zytomegalievirus-Erkrankungen bei Patienten mit einer **Human Immunodeficiency Virus** 1-Infektion. Schrappe-Bacher M; Steffen H M; Ollenschlager G; Salzberger B; Fatkenheuer G; Degenhardt S; Kruger G R. (Medizinische Klinik II und Poliklinik, Universitat Koln.) Klinische Wochenschrift, **(1989 Nov 3)** Vol. 67, No. 21, pp. 1108-15. Journal code: 2985205R. ISSN: 0023-2173. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: German.

AB Eleven **Human Immunodeficiency Virus** 1 (HIV1)-infected patients (10 male, 1 female; age 23-51 years (median 36); 10 male homosexuals, 1 IV drug abuser; WR3 1 patient, WR5 5, WR6 5) with intestinal Cytomegalovirus (CMV)-manifestations were compared with a group of 78 HIV1-infected patients in respect to their clinical, immunological and virus-serological data and the results of the histological and microbiological examination of endoscopically obtained biopsies. No differences were observed on age, sex, risk of infection, stage and immunological status. Bloody diarrhea was most important in discriminating CMV-colitis and non-CMV-related intestinal manifestations. Dysphagia and other symptoms occurring in patients with CMV-esophagitis were not able to predict CMV-esophagitis specifically. 6 of 11 patients with serological findings consistent with an active CMV-infection had no detectable CMV-manifestations; 6 of 11 patients with intestinal CMV-manifestations did not show serological findings suggestive of active CMV-infection. Ulcerative alternations of intestinal mucosa represent the most powerful indicator of intestinal CMV-disease in endoscopical examination. Only in two patients, ulcerative alterations were seen without diagnosis of CMV-disease being established. CMV was isolated in one of 11 patients, in two patients CMV was isolated from biopsies of unchanged mucosa. Simultaneous infection by HSV and CMV was detected in three patients, in one patient in the same localisation. Histology revealed **inclusion bodies** in 8 of 11 patients with intestinal CMV-disease, in no case **inclusion bodies** were seen without CMV-disease.

L48 ANSWER 25 OF 26 MEDLINE on STN

89309236. PubMed ID: 2501357. Cleavage of procaryotically expressed **human immunodeficiency virus** fusion proteins by factor Xa and application in western blot (immunoblot) assays. Ellinger S; Glockshuber R; Jahn G; Pluckthun A. (Institut fur Klinische und Molekulare Virologie, Universitat Erlangen-Nurnberg, Federal Republic of Germany.) Journal of clinical microbiology, **(1989 May)** Vol. 27, No. 5, pp. 971-6. Journal code: 7505564. ISSN: 0095-1137. Pub. country: United States. Language: English.

AB The proteins p15 and p24 of the **human immunodeficiency virus** (**HIV**) type 1 gag gene were expressed as fusion proteins in Escherichia coli for detecting antibodies against the acquired immunodeficiency virus by Western blot (immunoblot) analysis. These fusion proteins contain amino acids 1 to 375 of the E. coli beta-galactosidase linked to the viral protein(s) by a recognition sequence for the specific protease factor Xa. They are obtained in large amounts in insoluble **inclusion bodies**. To avoid ambiguous results caused by cross-reaction of sera with bacterial proteins in Western blots, we purified the recombinant fusion proteins and subsequently removed the bacterial part of the fusions by cleavage with factor Xa. The cleavage mixtures were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and blotted onto nitrocellulose membranes. The viral proteins obtained by this method did not contain any bacterial proteins or protein fragments. Thus, false-positive results in **HIV** Western blot analysis with bacterially expressed **HIV** proteins can be excluded with these purified recombinant viral antigens.

L48 ANSWER 26 OF 26 MEDLINE on STN

89174639. PubMed ID: 2647726. A 113-amino acid fragment of CD4 produced in Escherichia coli blocks **human immunodeficiency virus**-induced cell. fusion. Chao B H; Costopoulos D S; Curiel T; Bertonis J M; Chisholm P; Williams C; Schooley R T; Rosa J J; Fisher R A; Maraganore J M. (Biogen, Inc., Cambridge, Massachusetts 02142.) The Journal of biological chemistry, **(1989 Apr 5)** Vol. 264, No. 10, pp. 5812-7. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB A gene encoding a 113-amino acid, NH2-terminal fragment of CD4, rsT4.113, was constructed and expressed in Escherichia coli under the control of the tryptophan operon promoter. Following induction, rsT4.113 is produced at 5-10% of total E. coli protein, and it is found in **inclusion bodies**. The protein is purified in two steps under denaturing and reducing conditions. Solubilized rsT4.113 is first purified on a column of Q-Sepharose to remove low molecular weight contaminants and then purified to greater than 95% homogeneity by gel filtration. Renaturation of rsT4.113 is achieved at approximately 20% yield by dilution and dialysis. High performance liquid chromatography analysis of renatured rsT4.113 reveals a less than 15% contaminant of reduced protein. Purified and renatured rsT4.113 contains epitopes for both OKT4a and Leu3a, anti-CD4 monoclonal antibodies which block CD4-gp 120 association, but lacks measurable affinity toward a nonblocking anti-CD4 monoclonal antibody, OKT4. By comparison to a longer form (375 amino acids) of recombinant soluble T4 produced in mammalian cells that contains the entire extracellular domain, rsT4.113 has a comparable affinity for binding to OKT4a and Leu3a in a radioimmunoassay. Analysis of antiviral activity of rsT4.113 demonstrates that the E. coli-derived protein inhibits **human immunodeficiency virus**-induced syncytium formation with an IC50 of 5-10 micrograms/ml. These data demonstrate that the **human immunodeficiency virus**-binding domain of CD4 is localized within the NH2-terminal 113 amino acids of CD4 and is contained within a structure homologous to the kappa variable-like domain of immunoglobulins.

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(FILE 'HOME' ENTERED AT 11:43:32 ON 08 JAN 2007)

FILE 'USPATFULL' ENTERED AT 11:43:44 ON 08 JAN 2007

	E	KACZMAREK ALEXANDRA/IN
L1	1 S	E3
	E	KOPETZKI ERHARD/IN
L2	26 S	E3
L3	16 S	L2 AND (INCLUSION BODIES)
L4	3 S	L3 AND (INCLUSION BODIES/CLM)
L5	2 S	L4 NOT L1
L6	13 S	L3 NOT L4
L7	13 S	L6 AND AY<2003
	E	SCHANTZ CHRISTIAN/IN
L8	2 S	E3

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L9            0 S L8 NOT (L1 OR L2)
                E SEEBER STEFAN/IN
L10           6 S E3
L11           5 S L10 NOT (L1 OR L2)

     FILE 'WPIDS' ENTERED AT 11:49:15 ON 08 JAN 2007
                E KACZMAREK A/IN
L12          15 S E3
L13           1 S L12 AND (INCLUSION BODIES)
                E KOPETZKI E/IN
L14          30 S E3
L15           9 S L14 AND (INCLUSION BODIES)
L16           8 S L15 NOT L12
                E SCHANTZ C/IN
L17           5 S E3
                E SEEBER S/IN
L18          19 S E3
L19          18 S L18 NOT (L12 OR L14)
L20           2 S L19 AND (INCLUSION BODIES)

     FILE 'MEDLINE' ENTERED AT 11:53:20 ON 08 JAN 2007
                E KACZMAREK A/AU
L21          49 S E3
L22           0 S L21 AND (INCLUSION BODIES)
                E KOPETZKI E/AU
L23          19 S E3-E4
L24           0 S L23 AND (INCLUSION BODIES)
                E SCHANTZ C/AU
L25           2 S E3
                E SEEBER S/AU
L26         329 S E3
L27           0 S L26 AND (INCLUSION BODIES)

     FILE 'USPATFULL' ENTERED AT 11:55:20 ON 08 JAN 2007
L28         187 S (INCLUSION BODIES/CLM)
L29          98 S L28 AND AY<2002
L30           2 S L29 AND (MULTIPLE COP?/CLM OR MULTIPLE JOINED GENES/CLM)
L31          13 S (T1357 OR T-1357)
L32           9 S L31 AND AY<2003
L33           0 S L32 AND (T-1357/CLM OR T1357/CLM)
L34         458 S T-1249
                E BOLOGNESI D P/IN
L35          12 S E4
L36           0 S L35 AND L34
                E LAMBERT D M/IN
                E LAMBERT DENNIS M/IN
L37          22 S E3 OR E4
L38           1 S L37 AND L34
                E BARNEY S/IN
L39          22 S E7-E9
L40           1 S L39 AND L34

     FILE 'MEDLINE' ENTERED AT 12:05:13 ON 08 JAN 2007
L41       17324 S (INCLUSION BODIES)
L42        2797 S L41 AND PURIFICATION
L43        2197 S L42 AND PY<2002
L44           2 S L43 AND (MULTICOPY OR MULTIPLE COPIES OR MULTIPLE JOINED)
L45          18 S L43 AND REVIEW?
L46         969 S L43 AND (INCLUSION BODIES/AB)
L47           9 S L46 AND REVIEW?
L48          26 S L46 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)

=> s l46 and (fusion inhibitor? or antifusogenic)
        143766 FUSION
        848851 INHIBITOR?
           575 FUSION INHIBITOR?
                 (FUSION(W)INHIBITOR?)
             2 ANTIFUSOGENIC
L49          0 L46 AND (FUSION INHIBITOR? OR ANTIFUSOGENIC)

=> d his

     (FILE 'HOME' ENTERED AT 11:43:32 ON 08 JAN 2007)

     FILE 'USPATFULL' ENTERED AT 11:43:44 ON 08 JAN 2007
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                E KACZMAREK ALEXANDRA/IN
L1            1 S E3
                E KOPETZKI ERHARD/IN
L2           26 S E3
L3           16 S L2 AND (INCLUSION BODIES)
L4            3 S L3 AND (INCLUSION BODIES/CLM)
L5            2 S L4 NOT L1
L6           13 S L3 NOT L4
L7           13 S L6 AND AY<2003
                E SCHANTZ CHRISTIAN/IN
L8            2 S E3
L9            0 S L8 NOT (L1 OR L2)
                E SEEBER STEFAN/IN
L10           6 S E3
L11           5 S L10 NOT (L1 OR L2)

     FILE 'WPIDS' ENTERED AT 11:49:15 ON 08 JAN 2007
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L16           8 S L15 NOT L12
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L23          19 S E3-E4
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L49           0 S L46 AND (FUSION INHIBITOR? OR ANTIFUSOGENIC)

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ALL L# QUERIES AND ANSWER SETS ARE DELETED AT LOGOFF
LOGOFF? (Y)/N/HOLD:y
STN INTERNATIONAL LOGOFF AT 12:12:47 ON 08 JAN 2007